

A STUDY OF THE MAMMALIAN BREAST.

The Development of the Mammary Gland in the Mouse.

by

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of the University of Edinburgh, in the Faculty of Medicine.



..... we must never make experiments to confirm
our ideas but only **To my father.** that; one must
accept the results of experiments as they come,
with all their unexpectedness and irregularity.

Claude Bernard.

All the work described in this thesis was carried

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our ideas but simply to control them; one must
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My thanks are due first to the University and to the
Department of Anatomy for enabling Claude Bernard.
these studies.

All the work described in this thesis was carried out in the Department of Anatomy at the University of Edinburgh, during the period October 1963 to March 1966, whilst I was employed as a lecturer in the Department of Anatomy.

My thanks are due both to the University and to the Department of Anatomy for enabling me to carry out these studies.

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During the last 100 years very much advance in research
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Zoologists and anatomists of the late 19th century and
early 20th century devoted most of their attention to its
histological structure. Accounts of work on the mammary
gland during this period were given by Owen (1866) and
Barnard (1929).

The structure and role of ecto-epithelial cells in the
mammary gland, were considered in studies on lactation and
reviews on findings appeared at regular intervals (Simmons
1935; Berman, 1944; Richardson 1947; Sawyer 1949; Harkin 1954;
Richardson 1959 and Linzell 1952).

Several studies attempted to show that lactation was
dependent on a nervous mechanism. These arose from the
discovery of sensory nerves in the mammary and other
glands by Lewis (1911). Bach (1903) revised the work
on sensory nerves.

As a result of the work of Harkin (1955); Goltz and
Kendall (1936); Smith (1937); Ribbert (1936) and Linn-
Shannon and Shilling (1935) the role of a specific chemical
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During the last 100 years every new advance in research technology has been applied to the anatomy or physiology of the mammary gland. The aims of these investigations have changed during this period. As the evolutionary theory developed, interest in the mammary gland was predominantly focussed on its value as a means of species classification.

Zoologists and anatomists of the late 19th century and early 20th century devoted most of their attention to its histological structure. Accounts of work on the mammary gland during this period were given by Owen (1868) and Bresslau (1920).

The structure and role of myo-epithelial cells in the mammary gland, were considered in studies on lactation and reviews on findings appeared at regular intervals (Zimmerman 1898; Bertkau 1907; Lenfers 1907; Retterer and Lelievre 1911; Zimmerman 1927; Hamperl 1939; Babkin 1944; Richardson 1949 and Linzell 1952).

Several studies attempted to show that lactation was dependent on a nervous mechanism. These arose from the discovery of secretory nerves to the salivary and other glands by Ludwig (1851). Basch (1903) reviewed the work on mammary nerves.

As a result of the work of Eckhard (1855); Goltz and Ewald (1896); Routh (1897); Ribbert (1898) and Lane-Claypon and Starling (1906) the role of a specific chemical stimulus initiated by the presence of an embedded ovum in controlling the growth and activity of the mammary gland supplanted the importance of nervous control. This recognition/

recognition that "hormones" are intimately concerned with mammary development created a new field for investigation as part of the growing science of endocrinology. As purified hormonal extracts became available in the second and third decades of this century this field of study began to clarify the complex endocrine control mechanisms (Allen et al 1924; Parkes 1929; Koch 1937 and Allen et al 1939).

Another contribution to the study of the mamma was made by Lane-Claypon and Starling (1906) who developed a technique for the preparation of whole mounts of the mammary tree. This provided a means for studying changes in the natural shape and size of the mammary gland, and in suitable thin, flat glands (e.g. in the mouse and rhesus monkey) a means of making a quantitative analysis of these changes. This technique was soon adopted and improved by other workers (Ancel and Bouin 1911; Aberle 1934a; Gardner and Strong 1935; van Heuverswyn et al 1939; Folley et al 1939 and du Bois 1941, 1944). Their methods have been found particularly useful in recent years in the investigation of hormonal influences on normal and pathological mammogenesis.

Evidence of extensive endocrinological research on the mammary gland can be found in the literature in particular on the changes during pregnancy and lactation. Folley (1947, 1949) provided concise and comprehensive surveys of the endocrine and nervous control of lactation. His accounts were followed by further reviews by Pincus and Thimann (1948, 1950, 1955) and by Hadfield (1958).

The/

The demonstration of virus-like particles in the milk of certain strains of lactating mice with mammary tumours and the belief that these virus-like particles were the "mammary tumour inciters", further focussed interest on the mammary gland during pregnancy and lactation (Bittner 1939). This emphasis on pregnancy and lactation occurred to the exclusion of interest in the other phases of mammo-genesis. The significance of virus particles has been reviewed by Pitelka et al (1964).

The combination of knowledge gained from experimental animal research on normal and abnormal breast endocrinology, and the discovery that about half the cancers arising in the human breast are hormone dependent (Hadfield 1958), intensified studies on mammo-genesis during pregnancy and lactation.

The ability to transplant mammary tissues which had been applied to investigations on the effects of naturally occurring or artificially injected hormones by early workers (Stricker 1929), was also used to show that tumours could appear in transplanted mammary tissue (Shimkin et al 1946). The results obtained by transplantation under different conditions were reviewed by Hoshino (1962) in studies of normal mammo-genesis, and by Dao et al (1964) in studies on tumour induction in transplanted mammary tissue. The effects of transplanted tissue on the mammary duct system were studied by Argyris and Argyris (1960).

Improved electronmicroscopical and histochemical techniques allowed further progress in the study of the mammary/

mammary gland structure. Electronmicroscopic studies on normal cells by Bang et al (1956a), on myo-epithelial cells by Langer and Huhn (1958), on the breast structure during virginity, pregnancy and lactation by Hollmann (1959), Wellings, de Ome and Pitelka (1960) and on intracellular structures by Miyawaki (1965) were supported or combined with electronmicroscopical studies on malignant mammary tissue (Bang et al 1956a and 1956b; Mau and Rijssel 1961). Autoradiographic techniques have also been applied to studies of the mammary glands in mice. Again the emphasis is found to be on the adult breast and in particular in relation to its response to hormonal treatment (Traurig and Morgan 1964) or in relation to neoplastic disease occurring in it (Mise 1961).

The above summary shows that most intensive work on the mammary gland has been concerned with the problems of lactation and the normal and neoplastic ultrastructure and physiology of the mamma during post pubertal development.

Richardson (1947) in a general survey of studies on the mammary gland, pointed out that the mammary rudiments in the foetus and the newborn may possess inherent genetical variations in sensitivity to hormonal stimulation. He further stated that the stage of development at which this sensitivity was acquired in the foetus was unknown, and stressed the need for more experimental work on this problem involving quantitative measurements.

Balinsky (1950) examining the reviews of Turner (1939) and Speert (1948) on studies of the normal development of the/

the mammary gland in many different species, found that although the postnatal development and the factors that control its growth and differentiation had been investigated in great detail, the prenatal development was not so adequately covered. In particular, he noticed an absence of quantitative investigations into cell proliferation and the growth of the early rudiments of the mammary gland in mice. In a study on mouse mammary gland cellular proliferation during prenatal development, determined by calculating the mitotic indices and surface areas of the glands in serial section, Balinsky (1950) provided much of the necessary information.

The maintenance of blood cells, connective tissue and other tissues in tubes of serum or plasma by Loeb (1897) and the subsequent work by Harrison (1907) have been universally accepted as the origins of tissue culture. Comprehensive accounts of the history and progress of tissue culture techniques have been given by Paul (1965) and Willmer (1965). Growth of the mammary tissue elements in culture was first obtained by Maximov (1925) from explants of adult rabbit mammary gland fragments. Hardy (1949) noticed some mammary gland differentiation in a study of mouse hair follicle development in vitro and subsequently (Hardy 1950) described the development of the mammary apparatus from the anlage in cultures of parts of the ventral body wall of 10 to 13 day old embryos for periods of 4 to 25 days in a medium consisting of equal parts of adult cock plasma and chicken embryo extract. She noticed changes in the gross structure, histology/

histology and differentiation which were similar to those found in control embryos and postnatal mice up to the age of 7 days, and concluded that the early stages of the mammary development in the mouse were not primarily dependent on specific hormonal stimulation or other influences from different parts of the body. Lasfargues and Murray (1959) studying the effects of various hormones on the differential growth of the mammary gland stroma and epithelium from 10 to 15 day old mouse embryos, found that in a synthetic medium alone the abdominal epithelium penetrated the underlying connective and adipose tissues and formed a duct system. Prop (1959) examining the influence of progesterone on total mouse mammary gland cultures, refrained from using embryonic tissue because "independent development tendencies may obscure hormonal influences". Numerous studies followed these early investigations of the mammary gland in vitro, concentrating on the effects of hormones on cultured tissue in adult mice or rats. Surveys of work carried out during the last few years were given by Ranadive and Chapekar (1964) and Rivera (1964), (a and

From the above, it is evident that many aspects of mammo-genesis require elucidation. In particular, the embryo-genesis and early postnatal mammary gland development has been neglected. A quantitative analysis of this period in the mouse mamma of males and females would bring out more clearly the differential sexual development pattern. The value of being able to study the organ divorced from the organism under controlled conditions is obvious. The development of a culture technique in which the embryonic male and female glands can be cultivated and allowed to grow in an environment/

environment which closely resembles the environment in vivo would provide valuable basic information.

It is the purpose of this study to achieve these two objectives.

MATERIALS AND METHODS.

1. TEST ANIMALS.

The mice used in this study were from a substrain of the C57BL strain of mice as listed by the committee on standardized genetic nomenclature for mice. (Snell et al 1960).

This substrain, the C57BL-a^t/H strain was obtained from Dr. J.G. Howard, The Department of Surgical Science, University New Buildings, Teviot Place, Edinburgh 8.

The genetic history of this substrain is as follows:-

- I. The C57BL (Synonym C57 Black) strain. (Snell et al 1960)

Origin: Little, 1921, from heterozygous sib pair,

057 X 052, from Miss Lathrops stock.

Genetic constitution: (only mutant genes are listed) aa.

Inbreeding: (Number of generations of brother X sister inbreeding) X 60 (November 1959).

Characteristics: Very low mammary tumour incidence; no MTI (Mammary Tumour Inciter).

Resistant to mammary and ovarian tumour induction by chemical carcinogens. Sporadic

juvenile hair loss. Eye abnormalities in about 10% of animals; incidence varies in different

sublines. Large numbers of skeletal variants.

Various types of internal tumours.

Susceptible to lymphoma induction by X-irradiation.

- II. The C57BL-a^t strain. (Snell et al 1960)

Origin: Spontaneous mutation to a^t in C57BL/H strain in 6th generation at Harwell.

Genetic/

Genetic constitution: $a^t a \times aa$.

Inbreeding: $x ? + 13$ (November 1959)

Characteristics: The same as the C57BL strain.

III. The C57BL- a^t/H strain. (fig. 1).

This is a direct subline of the C57BL- a^t strain and was obtained from Harwell in January 1959. Since then it has been maintained by Dr. Howard and continuously inbred. Histocompatibility in this strain has been periodically tested for by reciprocal skin grafts from time to time. (Howard 1964).

2. MAINTENANCE OF ANIMALS.

The mice were maintained in metal mouse cages in temperature controlled, properly humidified animal quarters, on a diet of mixed grain (consisting of kibbled maize and wheat), rat cake diet No. 41, and water ad libitum.

3. CHOICE OF GLAND.

In all cases only the third thoracic or pectoral pair of glands were studied for the following reasons:

I. The two inguinal pairs lag behind the thoracic glands in their stage of development, and may show considerable variation from the normal pattern not only between animals from the same litter but also between glands from the same animal. This observation was first made by Schultze (1892) in the cat and fox, and later by Henneberg (1900) and Myers (1917) in the rat, and Turner and Gomez (1933) in the albino mouse. Strain variation in the stage of development of the three thoracic pairs of glands in male mice were reported by Richardson and Cloudman (1947) and Richardson F (1951 and 1953), but in all cases/

cases the third pair of thoracic glands showed, bilaterally, a greater degree of development than the other thoracic glands.

II. The caudal inguinal pair of glands can be absent in the male. In her study on nine major strains of male mice (excluding the C57BL strain) Richardson (1951) found no glands corresponding to the fifth pair in females. In earlier studies Schickele (1899) first noted the complete absence of nipples in the male and reported a considerable variation in the appearance of inguinal glands in both male and female rats and mice. Turner and Gomez (1933) found a similar variation in the inguinal glands of the albino mouse, while Richardson^F(1953) found that 50% or more of the mammary glands were absent or very small in the pure strains of mice she studied with the exception of the C57BL strain. (Her study considered the three thoracic and first inguinal pairs of glands only). Hadfield and Young (1956) reported that occasionally one of the second pair of thoracic glands may be congenitally absent in the albino mouse.

III. Whereas the thoracic glands grow laterally in a flat sheet away from the nipple area, the inguinal glands cluster round the nipple in a complex manner. (fig. 2) The uniplanar distribution of the duct systems of the thoracic glands allows for reasonably accurate area estimations to be made from two dimensional measurements. A quantitative analysis of developmental changes in the breast is therefore possible. For this reason, most quantitative studies of the breast are based on observations made on the thoracic glands.

Correlation/

Correlation of left and right glands:

In order to be able to use either the right or left gland as a control in experimental work on the breast, it had to be established whether there was good correlation between the right and left glands in the C57BL strain. Whole mounts were therefore prepared of the third thoracic gland pairs from 33 males and 33 females, aged 21 days. The number of end-buds in each gland was compared with that of the opposite side and used as an index of correlation.

4. CALCULATION OF ANIMAL AGE.

I. Prenatal animals.

The exact time of onset of pregnancy was controlled by taking the following precautions:

Siblings were separated after weaning at the age of 21 days. (Snell 1941) All females were kept in separate cages. Approaching maturity at the age of 39 days (Mirskaia and Crew 1930), all females were examined for the signs of onset of oestrus. Vaginal smears were carried out at 9.00 a.m. daily to detect the presence of heat. The technique used was a modification of the original method described by Stockard and Papanicolaou (1917) and later improved by Papanicolaou (1933). Smears were taken by ejecting a few drops of Sodium citrate into the vagina by means of a finely pointed pipette and immediately sucking it back into the pipette. The Sodium citrate with its cellular contents was then transferred to a glass slide and allowed to dry. All smears were stained with Wrights stain (Wright 1902/

1902; Nicholas 1942). The stained smears were examined and grouped according to the classification of Heape (1900) into pro-oestrus, oestrus, met-oestrus and di-oestrus. No female was smeared more than once in every 48 hours to prevent any possibility of false positive oestrus smears due to cornification of the vagina (Wade and Doisy 1935; Emery and Schwabe 1936). External signs such as, increased activity (Farris 1941), a lordotic copulatory response (Blandau, Boling and Young 1941), nipple proliferation (Aberle 1934b), and a gaping vagina with vulvar congestive swelling (Allen 1922; Long and Evans 1922; Clauberg 1931) although examined for, were not reliable as indicative of the onset of heat. When the vaginal smear showed the typical appearance found at oestrus (fig. 3), the female examined was immediately put in the male cage (Danforth 1916). Copulation almost invariably took place within 15 minutes of mating. The male and female were left together for a period of 6 hours before the female was again separated from the male. At the same time the presence of a vaginal mucous plug was noted (Sobotta 1895). After 48 hours another vaginal smear was carried out to confirm pregnancy. (fig. 4). Fertilization was estimated to have taken place from 2-6 hours after mating in every successfully established pregnancy. The time chosen for mating animals successfully (approximately 11.00 a.m.) was controlled by the following factors: (Table 1)

(i) FEMALE.

(a) The onset of heat occurs most commonly at night between 10.00 p.m. and 1.00 a.m. Occasionally it occurs between/

between 1.00 a.m. and 7.00 a.m. and in rare instances during the day. (Lewis and Wright 1935; Snell, Fekete, Hummel and Law 1940).

(b) After onset, heat is estimated to last for approximately 12 hours in mice. (Parkes 1928; Snell et al 1940).

(c) Ovulation occurs spontaneously during oestrus and may be either at the beginning of oestrus (Brambell and Parkes 1927; Lewis and Wright 1935; Snell et al 1940), or near the end of oestrus (Allen 1922; Togari 1927). The estimated time for the Macdowell-Bagg albino mice was between midnight and 3.00 a.m. (Snell et al 1940).

(d) Immediately after ovulation the eggs are found in the upper part of the oviduct. (Snell 1941).

(e) Maturation of the eggs takes from 4 to 15 hours after ovulation. (Kirkham 1907; Long and Mark 1911).

(f) Pincus (1936) estimated that mouse ova retained cytological normality for about 35 hours after ovulation whereas Charlton (1917) showed clear signs of degeneration in unfertilized tubal mouse ova by 2 days after parturition.

(11) MALE.

(a) After copulation the spermatozoa reach the ovarian end of the oviduct, where the mature eggs are lying, within 15 minutes.

(b) Penetration of the coverings of each egg resulting in fertilization takes approximately 2 hours. (Lewis and Wright 1935).

(c) The fertilizing ability of the spermatozoa is retained/

retained for about 6 hours after copulation. (Merton 1939).

II. Postnatal animals.

Daily examination of the cages containing pregnant females were carried out from 9.00 a.m. to 6.00 p.m. at hourly intervals from day 19 after mating. The normal range gestation period in the mouse is from 19 to 21 days. (Daniel 1910; Kirkham 1916). Cages were covered with metal lids and kept in a darkened room during the day in order to get more litters born during the day. (Danforth 1916; Merton 1938). Litters so discovered were up to one hour old and were regarded as newborn. The subsequent ages were calculated from this time.

5. SEXING OF ANIMALS.

I. Prenatal animals.

(i) 10 to 14 days of age.

Male embryos were recognised from the 10th day with identification of the differentiating testes by histological examination of the gonadal ridge in serially sectioned whole embryos. As the ovaries only become recognizable histologically at about 14 days, female embryos were selected by a process of exclusion up to that age. (Brambell 1927) (figs. 5 and 6).

(ii) 15 days to birth.

Sex was readily established by microdissection of the embryos. (Brambell 1927) (fig. 7).

II. Postnatal animals.

Sex was readily established in all postnatal animals by the external sex characteristics (Jackson 1912; Gruneberg 1943).

6. PROCEDURES.

I. Normal development.

This study consisted of:

(a) A histological study of serial sections of the developing mammary gland in male and female mice during embryonic life, at birth and during postnatal development up to the age of 21 days.

(b) A study of the overall changes in the size and shape of the mammary tree, taking place during this period as revealed by the analysis of whole mount preparations of the third thoracic gland pairs in both sexes. This provided measurements for a quantitative study of the sexual differences between the male and female glands.

As techniques differed in the prenatal and postnatal study, they are considered separately.

(i) Prenatal study.

The following age groups were studied.

	Serial sections		Whole mounts (No. of pairs)	
	M	F	M	F
11 days	+	+	-	-
12 days	+	+	-	-
14 days	+	+	-	-
16 days	+	+	+(1)	+(1)
17 days	+	+	-	-
18 days	+	+	+(10)	+(12)
19 days	+	+	+(10)	+(10)
20 days	+	+	+(10)	+(12)

At the desired stage of gestation the pregnant mice were killed/

killed with chloroform. A midline incision was made over the ventral abdominal wall exposing the uterus and its cornua. The embryos were extracted one by one and the membranes were carefully removed with the aid of a fine forceps. They were then immediately weighed and labelled and immersed in one of the following fixatives: (time shown after each fixative)

- (a) 10% neutralized formol saline - 24 hours.
- (b) Bouin - 24 hours.
- (c) Alcohol-formol-acetic - 24 hours.
- (d) Carnoy - 1 hour followed by 10% formol saline - 24 hours.
- (e) Susa - 24 hours.

Serial sections.

After fixation, dehydration was carried out slowly by placing the embryos in 50% ethanol for 12 hours, in 70% ethanol for 12 hours, in 96% ethanol for 3 hours and finally in three changes of absolute ethanol for a total time of 3 hours. With the larger embryos (18-20 days) the period in absolute ethanol was increased to 4 hours. (Dehydration of embryos fixed in Susa or Carnoy was started in 96% ethanol). In all cases, vessels were placed in a tumble action agitator to ensure adequate dehydration. The tissues were then double-embedded using a modification of the method employed by Brain (1949).

After final dehydration the embryos were placed in equal parts of absolute ethanol and methylsalicylate for 2 hours and then transferred to methylsalicylate alone until clear. They were then immersed in a 1% solution of celloidin in methylsalicylate/

methylsalicylate for 48 hours. The tissues were then treated in benzene for 6 hours and after 1 change in a benzene-paraffin mixture at 37°C for 1 hour finally embedded in 56°C paraffin wax. Blocks of tissue were serially sectioned at 8 or 15 microns, mounted on albumenized glass slides and dried in a 37°C oven before staining. Sagittal and coronal sections were routinely stained by one of the following methods: (Fixative used indicated in each case)

(f) Harris haematoxylin and eosin as a general stain.

(10% formol saline; Bouin; A.F.A.) (Carleton and Drury 1957).

(g) Mallorys stain - Lebenden modification for fibrous tissue and collagen. (10% formol saline; Bouin; A.F.A.) (Cullings 1957).

(h) Massons stain - Foot and Goldner modification for (a) fibrous tissue and collagen (10% formol saline; Bouin; A.F.A.)

(b) Myoepithelial cells. (Susa) (Edwards 1950).

(i) Peters silver proteinate method for reticulum and nervous tissue. (Bouin; A.F.A.) (Peters 1958).

(j) Romanes silver method - Linzell modification for myo-epithelial cells. (A.F.A.; Carnoy) (Linzell 1952).

Whole mounts.

From the age of 16 days whole mount preparations were made using a modification of the original technique described by Lane-Claypon and Starling (1906).

After fixation in 10% neutralized formol saline the gland bearing pelt was removed from the thoracic region of the carcass by/

by carefully incising it from the base of the skull to the mid-lumbar region and carrying the incision further around the ventral abdominal wall below the level of the umbilicus and along the inferior margin of the mandible. The fore limbs were then cut off at the level of the elbow joints and the pelt removed by holding the body firmly and the skin lightly and pulling the body away from the skin. Special care was taken not to damage the gland bearing areas near the axillae while stripping the pelt off. The whole pelt was then dehydrated by placing it in 50% ethanol for 4 hours, in 70% ethanol for 4 hours, in 96% ethanol for 2 hours and finally in three changes of absolute ethanol for a total time of 1 to 3 hours depending on the size of the pelt. It was then cleared in xylene until completely transparent. This usually took from 2 to 4 hours. The pelt was then rehydrated by reversing the process in graded ethanols and then washed in running tap water for 1 hour. It was then stained in Weigert iron haematoxylin for 4 hours. Differentiation was carried out under visual control, by placing the overstained pelt in a petri dish of a convenient size to fit the stage of a dissecting microscope, in a 2% solution of HCl in 90% ethanol.

After differentiation the pelt was again dehydrated in graded ethanol solutions and finally cleared in xylene. (Whereas other recent workers (Hadfield and Young 1956) stained their specimens directly after fixation and then dehydrated and cleared it before mounting, it was found in this study that considerably better staining reactions were obtained/

obtained by the above process). Wedge-shaped blocks containing the gland rudiments of the third thoracic pair of glands were then cut out of the pelt with the apex of the wedge at the nipple end of the gland in females and the proximal end of the gland in males. Blocks were then mounted between 2 glass slides in D.P.X. Shadowgraphs were prepared of each whole mount by projecting an image of the whole mount on to a screen at a magnification of X100 or X50 depending on the size, and tracing the exact outline area covered by the gland image. (fig. 8 and 9) From each whole mount the following measurements were obtained:

The total number of end-buds present.

The total duct length. This was measured in millimeter with the aid of a map measure which was run along the centre of each duct. A similar technique was used by Nicoll (1965) who made his measurements on a photographic print at a magnification of X 14.3 which he regarded as sufficiently high to eliminate inaccuracies in technique. In this study a magnification of X 400 was used which made it very easy to run the map measure exactly along the centre of each duct and ensured accurate results.

The outline gland area. This was measured in sq.% and was calculated by casting the gland image on to % squared graphic paper at a magnification of X 316.2 and then counting the number of % squared blocks covered by the image. The total number of % squared blocks counted divided by the area magnification (i.e. X 100,000) gave a close approximation of the true glandular outline area. Border line squares were included/

included or excluded in this count by observing whether the centre square in each 25 sq.% was inside or outside the exact outline of the gland.

The total number of end-buds and the total duct length were then related to age and body weight separately in both sexes and each relation was plotted graphically.

The gland areas were first related to age in both sexes and then used to compare the dynamics of mammary growth and bodygrowth in males and females by means of the method of relative growth analysis of Huxley (1932): Changes in the mammary gland area (A) were compared with changes in (body weight (W)) ^{$\frac{2}{3}$} , a function of the body surface area (Meeh 1879), using the allometric equation $\log A = \log B + (\log(W)^{\frac{2}{3}})$ (Cowie and Folley 1947; Cowie 1949; Silver 1953a,b). The comparison was plotted graphically with data for mammary gland areas expressed in square microns, and for body weights in mgm. Common logarithms were used in the analysis and the regression lines were fitted by the method of least squares. The validity of this procedure when logarithmic data are involved has been discussed by Cowie (1949).

(ii) Postnatal study.

The following age groups were studied:

	Serial sections		Whole mounts (No. of pairs)	
	M	F	M	F
24 hours	+	+	+(7)	+(14)
4 days	+	+	+(8)	+(12)
6 days	+	+	+(15)	+(12)
8 days	+	+	+(10)	+(18)
10 days/				

	Serial sections		Whole mounts		(No. of pairs)
	M	F	M	F	
10 days	+	+	+(12)	+(15)	
14 days	+	+	+(19)	+(15)	
21 days	+	+	+(33)	+(18)	
42 days	+	+	+(1)	+(1)	

The initial procedure was identical for both histology and whole mount preparations:

After careful weighing and labelling, all animals were sacrificed by chloroform anaesthesia. In the case of mice with hairbearing skin, usually of 10 days or more of age, the pelt was treated with barium sulphide for 1 minute and then washed in tap water to remove all excess hair (Cole 1934). The pelt was then removed in toto by using a modification of the technique of Hadfield and Young (1956):

An incision was made down the midline of the back from the vertex of the skull to the root of the tail. The tail and the fore and hind limbs were then cut off at the level of the elbow and knee joints. The pelt was then removed in the same way as in the prenatal study. After removal from the carcass the pelt was carefully rolled out, with its deep surface uppermost, and pinned down on a paraffin wax slab. The wax slab with its pelt was then labelled and immersed in fixative. For all whole mount preparations the pelt was fixed in a 5% solution of glutaraldehyde in 0.1 M Sørensen phosphate buffer at a pH of 7.2 (Sabatini; Bensch and Barrnett 1963; Sørensen 1912). Fixation of these preparations were complete within/

within 4 to 6 hours. For the histological serial sections, the same fixatives as used in the prenatal study were employed.

Serial sections.

After fixation the pelt on the wax slab was examined under a binocular dissecting microscope using a magnification of 15 diameters and transmitted light from an intense light source. In females over 6 days of age it was not difficult to pick out the third pair of nipples and their mammary duct systems. However, in the case of the male glands, where there are no nipples, and younger females where the nipples are not so obvious, it was more difficult to locate the glands and blunt dissection frequently resulted in loss or damage to the glands. A drop of 1% methylene blue in saline over the axillary area however quickly stained the glandular epithelium deep enough to reveal ^{its} ~~their~~ exact positions and extent. (fig. 10). After identifying the glands, blocks were cut out of the pelt with the gland contained in its fat pad deep to the panniculus carnosus attached to its deep surface. The size of these blocks varied with the age and sex of the animals but in each case was wedge shaped, with the apex of the wedge at the nipple end of the gland in females and at the proximal end of the gland in males, as in the prenatal study. The blocks of tissue were then dehydrated by passing ~~it~~ through ascending strengths of graded ethanol solutions. The times varied according to the size of block involved but in all cases dehydration was complete within 12 hours. After dehydration the blocks were first cleared in benzene for 1 to 4 hours, and then placed in a 50% solution of benzene in 56 C. paraffin wax at/

at 37 C. for 12 hours before final embedding in 56 C. paraffin wax. The further procedure was identical with that of the prenatal study.

Whole mounts.

After fixation in 5% glutaraldehyde, the pelt on the wax slab was examined under a binocular dissecting microscope. The skin was then lightly stained with a few drops of a 1% solution of methylene blue in saline and the gland bearing fat, aponeurosis and muscle were then separated from the hairbearing skin in one sheet by careful dissection and transferred to 50% ethanol in a petri dish of convenient size to fit the stage of the dissecting microscope. The tissue was then further processed using a similar technique as in the prenatal study. It was found, however, that Harris haematoxylin stained the postnatal preparations more quickly and just as satisfactorily as the prenatal glands stained with Weigert haematoxylin. Shadowgraphs were prepared of each whole mount as before using a magnification of X 25 and tracing the exact outline of the glandular duct system. Whole mount shadowgraphs were also prepared of the thoracic glands of a randomly selected male and female mouse aged 42 days to give some idea of the ultimate overall development at puberty. The female selected was found to be in oestrus by vaginal smear and this was confirmed by the complex gland structure as shown in the whole mount shadowgraph. (fig. 11). From each whole mount preparation the total number of end-buds, the total duct length and the outline gland area was calculated using the same techniques as for the prenatal material. The three measurements were then/

then analysed in the same way as in the prenatal study.

II. Experimental development.

(1) Introduction.

In order to study the extent to which the mammary glands of the male and female mouse of the C57BL^a/H strain depend on hormonal or other stimuli (either maternal, foetal or both) for their differential sexual pattern of development, an organ culture method was developed. Before the effects of any added hormones or other factors (vitamins, enzymes etc.) can be assessed quantitatively, a quantitative analysis of growth in vitro in cultures under carefully controlled conditions, imitating as closely as possible the environment of the gland in vivo, must be carried out.

Organ culture is particularly suited to embryonic material because as Trowell (1959) pointed out:

(a) Whole organs from embryos are so small that they can be cultured entire.

(b) Embryonic tissues in contrast to mature tissues, are remarkably resistant to the rather anoxic conditions prevailing in organ culture.

(ii) Apparatus.

Many types of organ culture chambers have been devised over the last 15 years. In most, special emphasis was put on the need to maintain normal architecture while allowing organised growth and development of tissue in culture, and the need for good visual and photographic recording facilities to illustrate the organ growth and behaviour (Hu et al 1951; Mackanass 1952; Christiansen et al 1953; Buchsbaum/

Buchsbaum and Kunz 1954; Rose 1954; Dick 1955; Harris 1955; Richter and Woodward 1955; Pulvertaft et al 1956; Pipkin and Mack 1958; Constable and Moffat 1958; Cruickshank et al 1959). Although all of these were intended to be simple in design and use, some had serious disadvantages as was pointed out by Dick (1955) and Cruickshank et al (1959).

Trowell (1952; 1954; 1959) solved many of the problems by designing a chamber with a built in gas reservoir and the advantage that the medium could be sampled, changed or added to without disturbing the cultures.

Hardy (1949; 1950) cultured the ventral body wall from mouse embryos by the watch-glass method of Fell and Robison (1929). Prop (1959) first cultured whole mammary glands of adult mice in small rings with paraffin cups containing the medium, but later (1960; 1961a; 1961b; 1961c; 1963) changed to a modification of the method developed by Trowell (1959) with success.

The culture chambers used in this study were made of perspex and were modelled on a modification of the type 2 chamber designed by Trowell (1959). (fig. 12). The purpose of these chambers were to be simple in design with the minimum of preparation required before use, to house several cultures in medium with a reservoir of gas to satisfy the needs of the growing tissues for at least 24 hours, and to allow changing of the medium without disturbing the cultures.

The chamber (fig. 13) has outside dimensions of 6.6% X 6.6% X 4.6%. The culture medium is contained in a glass petri dish (fig. 13c) with an outside diameter of 4.0% and a height/

height of 1.5%. The petri dish is supported by a perspex shelf (fig. 13s) which is attached to the inside of the chamber roof. This shelf is provided with ventilation holes (fig. 13v) to allow proper ventilation and gaseous equilibrium of the cultures with the main body of the container. Inside the petri dish stands a square stainless steel wire grid (fig. 13g) 2.5% X 2.5% which is supported by 1.0% legs made by bending the ends downwards. On top of the grid is placed a piece of lens paper (Green C105) also 2.5% X 2.5% in diameter. The cultures were explanted on the lens paper. This method of explantation has been extensively used (Trowell 1949; 1955; 1959; Seaman 1956; Seaman and Strahl 1956; McKenna and Stevens 1957; Stevens and McKenna 1957). A floating lens paper method originally described by Chen (1954) and applied by many workers (Richter et al 1956; Richter, Ritcheson and Cloud 1957; Richter, Cloud and Ritcheson 1957), was tried but like Trowell (1959) it was found that ten or more cultures could not be supported on the floating lens paper and therefore it had to be laid out on a grid. Culturing for only 5 days never led to troublesome adherence of cultures to the paper. (Schaffer's (1956) cellulose acetate fabric was tried but the presence of "ghost" lines on the cultures after fixation and dissolution of the fabric distorted whole mount preparations of the developing glands).

Covering the floor of the chamber is a square pad of felt (fig. 13f) 0.5% thick. This was moistened with sterile water during use of the chamber to retain a moist atmosphere which improves conditions for growth of cultures (Fell 1965).

A/

A gas inlet opening (fig. 13 I), sealed by a tight fitting No. 17 Suba-seal rubber stopper is situated in the bottom rear corner of the chamber just above the level of the felt square. This inlet also served the purpose of allowing re-moistening of the felt square.

The gas outlet opening (fig. 13 O), also sealed as above is situated immediately above the petri dish in an eccentric position. This outlet also served the purpose of allowing addition to or changing of the medium.

The chamber is opened by a sliding door (fig. 13 D). During cultivation this door was completely sealed around the outside edge by means of beeswax (Cruickshank et al 1959).

The total gas space of the chamber with the cultures in situ is 120cc. Ten circular cultures each of less than 4 % in diameter and less than 1 % in thickness could be cultured together in one chamber. These cultures have an estimated oxygen consumption of approximately 1.5cc. per 24 hours and a carbon dioxide production of approximately 2.0cc. per 24 hours (Trowell 1959). It should therefore only be necessary to re-gas every 72 hours, however, as the medium was changed every 24 hours (see procedures) re-gassing was carried out at the same time.

Trowell (1959) using the formula $r = 6CD/A$ (where A= oxygen consumption in cc. per cc. of tissue per minute, C=external oxygen concentration in atmospheres, D=the diffusion constant of oxygen, and r=cm.) (Gerard 1931), calculated that the limiting size of a spherical culture of lymphoid tissue was a diameter of 1.4%. In practice, he/

he found it to be slightly larger, namely 2% in diameter.

Skin with a relatively low oxygen consumption has a bigger limiting size (Krebs and Johnson 1948).

In this study it was found that cultures of up to 4% in diameter survived quite satisfactorily provided they were grown in a mixture of 5% carbon dioxide in oxygen. 5% carbon dioxide in air, as suggested by Trowell (1965), was found successful only in much smaller cultures and was therefore not used.

The apparatus was cleaned and sterilized immediately before use as follows:

The perspex culture chambers were rinsed in 96% ethanol and kept in a sterilized hood to dry.

The glass petri dishes and stainless steel wire grids were placed in boiling water for 10 minutes and then transferred to the sterilized hood.

The lens papers were treated by a modification of the technique followed by Chen (1954). After initial washing in two changes of ether for 2 hours, the papers were rinsed in three changes of absolute ethanol for 2 hours and washed in glass distilled water overnight. Immediately before use, the papers were dried in an oven at 80°C.

The felt pad was sterilized in an autoclave and kept in the sterilized hood.

During and after cleaning and sterilization all the articles were handled with sterile forceps using an aseptic technique.

(iii) Medium.

The/

The basic medium used for culture was the synthetic protein free medium T8 developed by Trowell (1959). (fig. 14). This was supplied by Difco in unit strength in a sterile container. When not in use, it was stored for not more than 2 months before use at a temperature of 0°C. (Paul 1965).

(iv) Embryo extract.

(a) Preparation.

A homogenate extract of mouse embryos was prepared by using a modification of the technique described by Paul (1965):

Pregnant mice were sacrificed by chloroform. Embryos were immediately extracted and after careful removal of the membranes thoroughly rinsed in Hanks balanced salt solution to remove all blood. Each embryo was then examined under a binocular dissecting microscope and the sex was established by the external appearance of the genitalia and confirmed by micro-dissection of the reproductive organs as in the normal development study. Male and female embryo homogenates were then separately prepared in a tube homogeniser. An equal volume of Hanks balanced salt solution was then added to the pulp and stirred with a sterile glass rod. The mixture was then transferred to a centrifuge tube and left to stand at room temperature for half an hour before centrifuging at 2000g for 20 minutes. The supernatant was then carefully removed and distributed into labelled test tubes. The extract was used fresh where possible but if storage was required, it was kept for not more than 8 days at -20°C. Stored extract was re-centrifuged after thawing immediately before use.

(b) Age of embryo extract used.

Gaillard/

Gaillard (1935) using either embryo extracts or postnatal animal serum as a cultivation medium, found that the differential growth of tissues in these media was dependent on the age of the embryos or postnatal animals from which the extracts or serum was obtained. In view of this finding, the embryo extract added to the Trowells T8 medium in this study was changed on successive days by older embryo extract. Therefore, the explants from 16 day old embryos (male and female) were cultivated for the first day in Trowells T8 medium + extract from 16 day old embryos and then successively in fresh medium + extracts from 17, 18, 19 and 20 day old embryos. In this way, an attempt was made to imitate closely the glandular environment during normal embryogenesis in the cultivation medium.

(c) Concentration of extract in medium.

Carrel and Ebeling (1923) and later Fischer and Parker (1929) and Zakrewsky (1929) showed a difference in the growth promoting effects of different concentrations of extracts from embryos of any one age in the medium on various fibroblast cells. Gaillard (1935) investigating this further, showed that tissue extract and serum obtained from embryos or postnatal animals of the same ages had an equal effect on the growth of heart fibroblasts in culture. He also found that cultures of osteogenetic cells in various concentrations of chick embryo extract or various concentrations of adult hen serum over a range of ages of extract or serum from 4 day chick embryos through to adult hen serum, grew best in an optimum concentration for each age group tested. The osteogenetic cells cultured first in 4 day old chick embryo extract, showed optimum growth at/

at a concentration of 50% embryo extract in the medium. At later stages it required a successively lower concentration of extract for optimal growth until it reached the 15 day old extract where the optimum growth promoting concentration was only 1.56%. After 15 days the optimum concentration of extract rose again over successive ages up to birth, and then the osteogenetic cells grew optimally in 50% chick serum or 50% adult hen serum.

In this study, no attempt was made to find the optimum concentrations of embryo extract for growth in the explants over the 16 to 20 day period of embryonic development. Hardy (1949; 1950) cultivated her explants in a mixture of equal volumes of adult cock plasma and chicken embryo extract and Prop (1961a; 1961b; 1961c; added 5% human male serum to his media for the study of postnatal mammary glands in culture. Throughout the concentration of embryo extracts used in this study was 25% of the final medium.

(v) Maternal serum.

(a) Preparation.

Serum was prepared from pregnant maternal blood by using a modification of the technique described by Paul (1965):

Maternal blood was obtained by sterile venepuncture of the tail vein of pregnant mice at the desired stage of gestation immediately before sacrificing for the delivery of the embryos. Serum was then prepared by permitting the whole blood to coagulate and sinerese and thereafter removing the exuded serum. It was then filtered with sterile precautions and stored in labelled bottles for less than 8 days at -20°C .

(Paul/

(Paul 1965).

(b) Age of serum used.

In the same way as with the embryo extract, the pregnant maternal serum added to the medium was changed on successive days by serum obtained from pregnant mice at progressively later stages of gestation. Therefore, the explants from 16 day old embryos (male and female) were cultivated for the first day in medium + serum obtained from a pregnant mouse at day 16 of gestation and then successively in fresh medium + serum from females at 17, 18, 19 and 20 days of gestation.

(c) Concentration of serum in the medium.

As in the case of the embryonic extracts maternal serum was used at a concentration of 25% of the final medium throughout.

Cultures.

In all cases a skin flap from the ventral body wall of 16 day old male and female embryos with the third thoracic mammary gland rudiment attached to its deep surface, was explanted under different conditions. Male and female gland rudiments were cultivated separately and together in all media to establish whether this would affect the growth response.

The following cultures were carried out:

In Trowells T8 synthetic medium only.

	No. of glands	Serial sections	Whole mounts (No)
Males alone	9	+	+(8)
Females alone	11	+	+(10)
Males with females	5	+	+(4)
Females with males	5	+	+(4)

In/

In Trowells T8 + Male embryo extracts + Maternal serum.

	No. of glands	Serial sections	Whole mounts (No)
Males alone	8	+	+(7)
Females alone	7	+	+(6)
Males with females	5	+	+(4)
Females with males	5	+	+(4)

In Trowells T8 + Female embryo extract + Maternal serum.

	No. of glands	Serial sections	Whole mounts (No)
Males alone	9	+	+(8)
Females alone	9	+	+(8)
Males with females	7	+	+(6)
Females with males	7	+	+(6)

Where possible all procedures were carried out under a sterilized hood to minimise contamination with dust from the atmosphere. The hood consists of a box made of wood with a glass top, with outside dimensions of 80% X 20% X 23% (fig. 15). The wood is lined on the inside with hard board. A binocular dissecting microscope is attached to the box with the stage and mirror inside the box below the glass top and the eye pieces, objectives and adjustment outside. Illumination comes through a window in the back wall. The front open end of the hood is provided with a polythene curtain so that both hands can be inside the box while the curtain prevents contamination of apparatus and cultures by breathing etc. Immediately before use the inside of the hood including the stage, base and mirror of the microscope is wiped with 96% ethanol and ether.

Preparation of the culture chamber.

The/

The glass petri dishes each containing a grid were set out in front of the empty chambers. The required volume of medium was made up, allowing approximately 15 cc. for each chamber. The medium was then slowly pipetted into each dish until it was spread completely across the underside of the grid. (This amount of medium was sufficiently to reach and wet the lens paper with the cultures therefore lying on the surface of the medium).

Preparation of the explants.

Pregnant mice were sacrificed at day 16 of gestation and briefly dipped in 96% ethanol to sterilize the pelt. The abdomen was then opened and the skin widely reflected. The uterus with its cornua was then carefully dissected out and immediately immersed in sterile Hanks balanced salt solution to wash off blood (Paul 1965). The embryos were then removed one by one and transferred to the hood in a petri dish. Sex was established by examination of the external genitalia and confirmed by microdissection as in the normal development study. (Vaginal bleeding described by Hardy (1949) was not found useful as an aid in sexing at this age). A circular flap of skin of less than 4% in diameter, overlying the position of the third mammary rudiment was then dissected from the body with the aid of fine cataract knives (Hardy 1949; 1950). (By staining and measuring the glands in the pelts from several embryos aged 16 days, it was possible to predict quite accurately the exact location of the third mammary rudiments in these embryos). The flaps were then transferred to the lens paper on the grid and arranged in an order which was/

was recorded so that each culture was individually identifiable. The bottom right corner of the lens paper also cut off to ensure orientation. The glass dish with its cultures in situ were then placed on the shelf in the chamber and the sliding door closed and sealed with beeswax.

Gassing of the chambers.

The chambers were gassed by passing a 5% carbon dioxide in 95% oxygen mixture through the chamber for 15 minutes at a flow rate of 5 to 10 cc. per minute. The gas mixture was fed into the chamber through the inlet valve by means of a guage 1 hypodermic needle which was attached to a polythene tube leading from the gas cylinder. At the same time, air and gas were allowed to escape through the outlet valve by means of a guage 1 hypodermic needle. After 15 minutes the gas flow was stopped and both needles withdrawn.

Cultivation of cultures.

The chambers were placed in an incubator and kept at a thermostatically controlled temperature of 37°C . Variation in the temperature was only of the order of plus or minus 1.5°C . over 24 hours. At no time was the temperature allowed to rise above 38.5°C . (Paul 1965). The cultures were cultivated for periods of 24 hours before the medium was changed and the chamber re-gassed. Changing of the medium was accomplished by first removing all medium from the petri dish with a hypodermic needle and syringe and then replacing it with the fresh medium, again ensuring that the volume was sufficient to spread out along the under surface of the lens paper. The whole procedure took approximately 20 minutes to complete including

15 minutes of re-gassing to wash out any accumulated carbon dioxide.

After 5 days of culture, the chambers were opened and the lens papers with the cultures in situ were removed from the dish. The lens papers were then cut into pieces with one culture on each piece and the pieces immersed in 10% formol saline, each in a separately labelled bottle for later identification. Fixation was complete within 4 hours. The explants were then washed in distilled water and dissected off the lens paper under microscopic control. In many cases the tissue fragments had already separated from the paper during fixation or washing. Using a similar technique as in the normal development study, whole mount preparations and shadowgraphs were prepared of the glands cultivated. From each chamber one specimen of each sex was embedded in paraffin wax and serially sectioned at 8 microns for histological examination. All sections were stained with haematoxylin and eosin. From each shadowgraph preparation the following measurements were obtained:

The total number of end-buds.

The total duct length in %.

The area enclosed by the outline of the gland in sq. %.

All the measurements were compared with those of the normal gland at the age of 20 days of embryonic life.

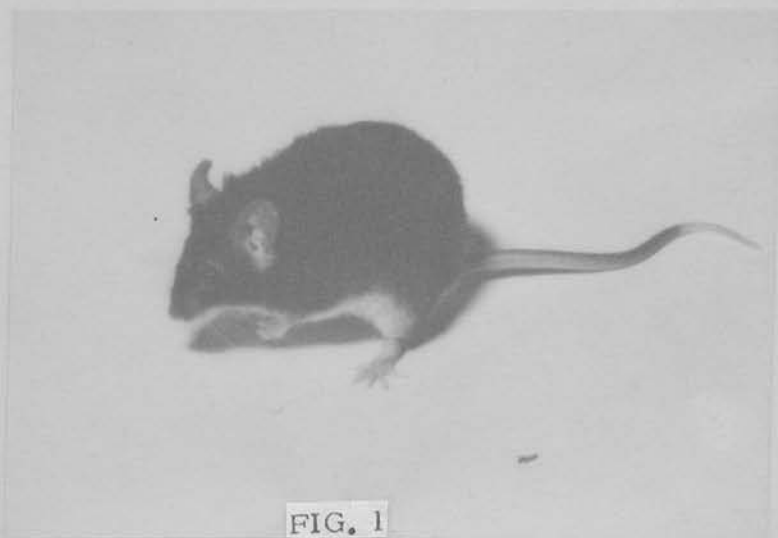


FIG. 1

Adult female mouse of the C57BL-6/J strain.

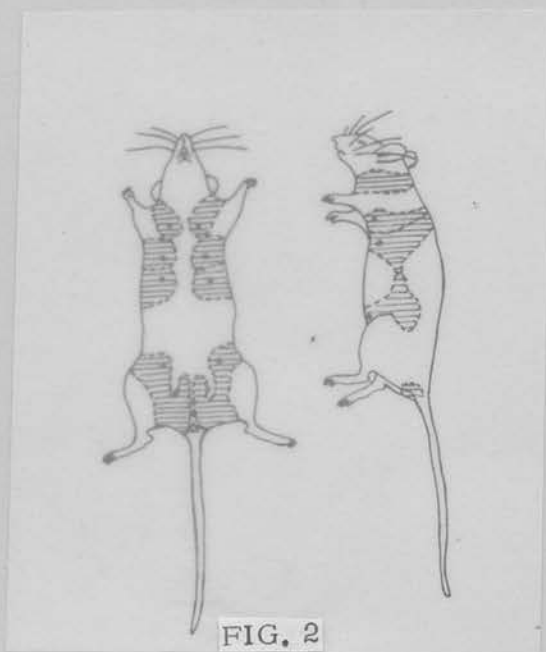


FIG. 2

Diagram of the ventral and lateral aspects of the female mouse illustrating the position of the nipples and the maximum extent of the mammary glands. (After Cloudman, 1941)

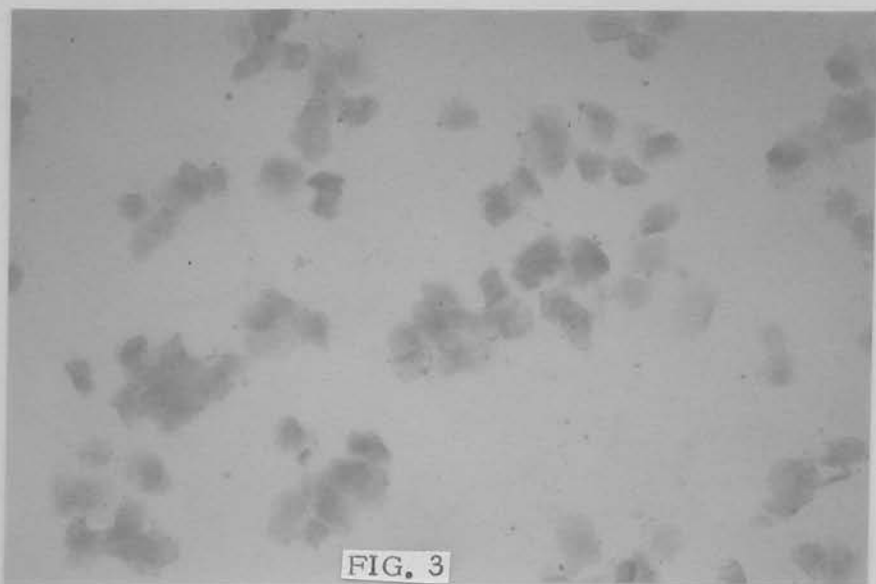


FIG. 3

Vaginal smear during oestrus. Characterised by the presence of only non-nucleated cornified epithelial cells. (After Heape 1900)
Wright. X 132

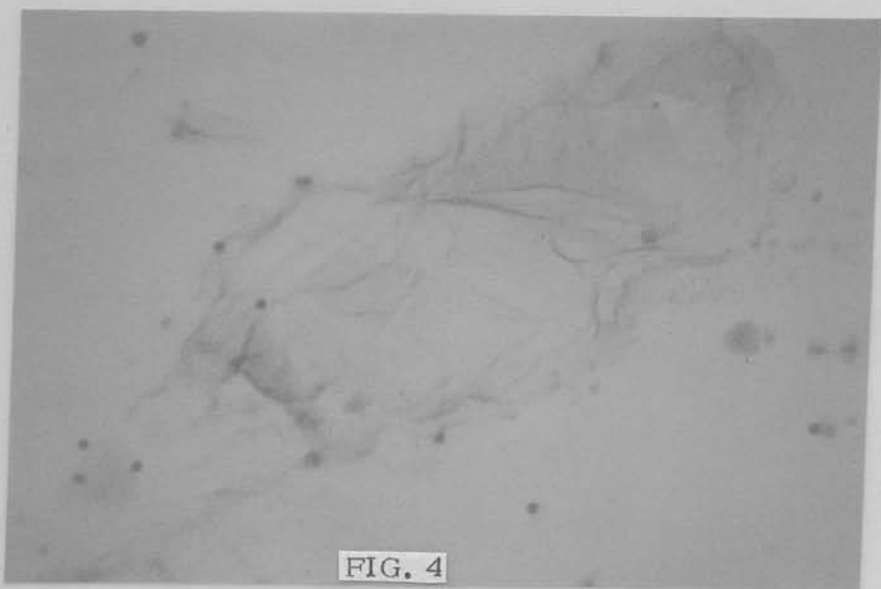


FIG. 4

Vaginal smear during pregnancy. Characterised by the presence of large quantities of mucous, some leucocytes and some epithelial cells with pale staining nuclei. (After Heape 1900)
Wright. X 276

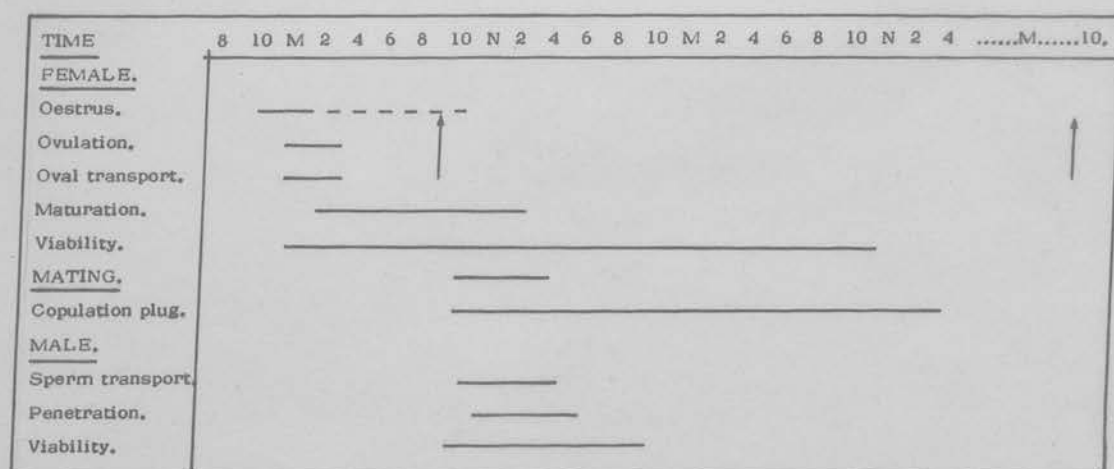


TABLE 1

COMPOSITE TABLE OF FACTORS CONTROLLING THE ONSET OF PREGNANCY IN MICE.

The arrows indicate timing of vaginal smears.

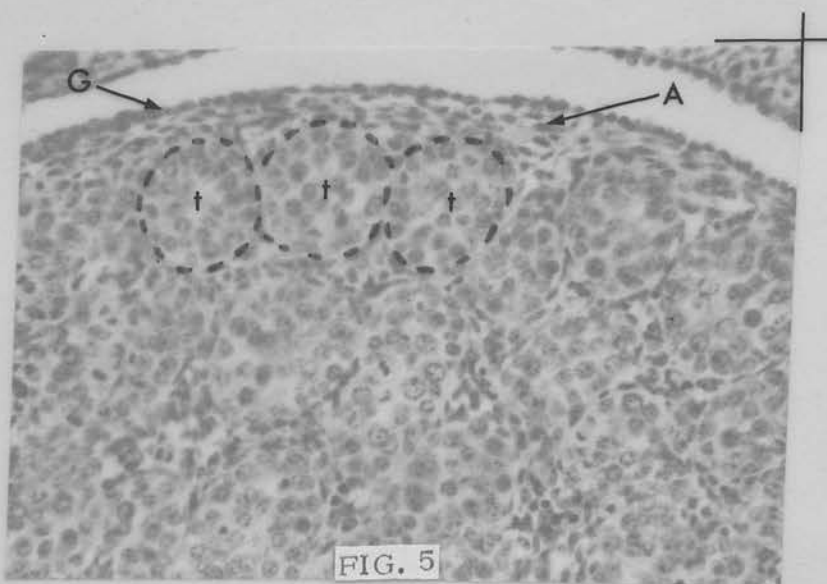


FIG. 5

Transverse section through testis of a thirteen-day embryo. Spermatic tubules are well formed and are separated from the germinal epithelium by an easily distinguishable tunica albuginea.

Haematoxylin and eosin. X 330

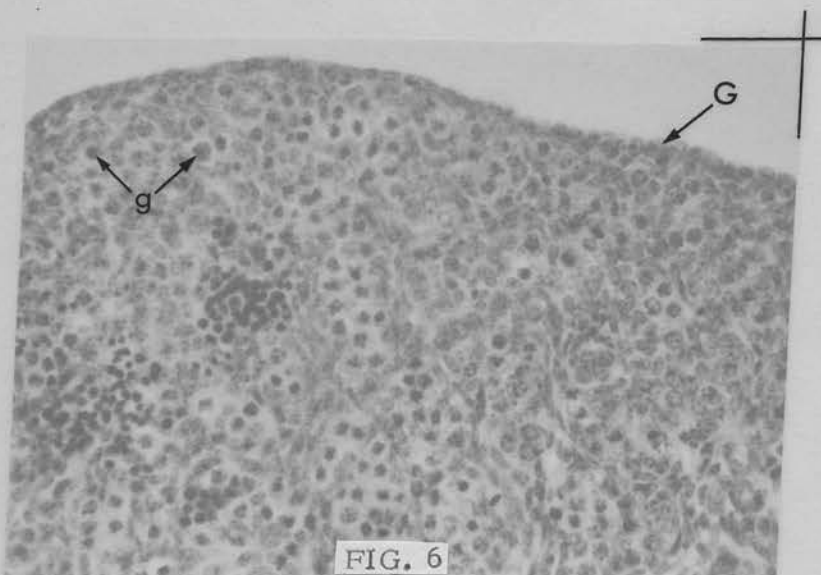


FIG. 6

Transverse section through ovary of a thirteen-day embryo. There is no conspicuous tunica albuginea beneath the germinal epithelium. Germ cells are scattered in the mesenchymal stroma.

Haematoxylin and eosin. X 330

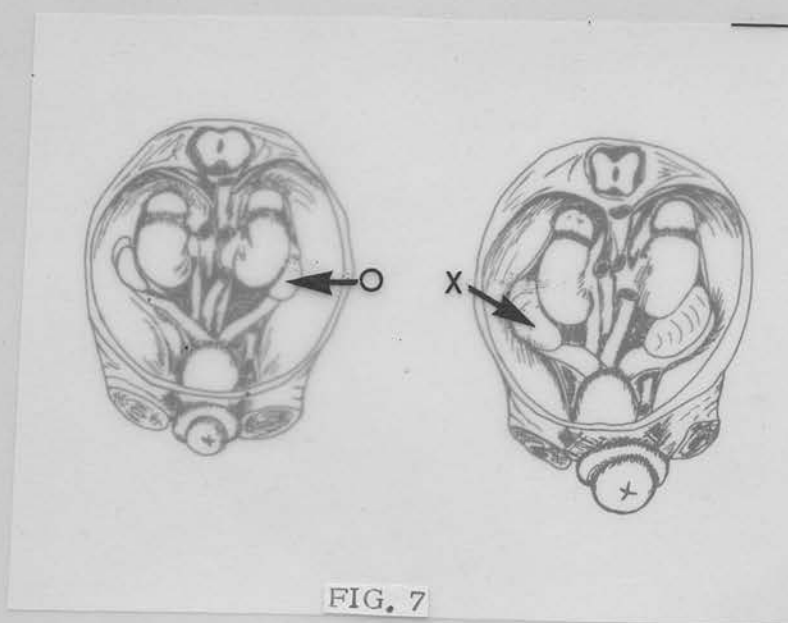


FIG. 7
Microdissections of fifteen-day male and female embryos. (After Brambell, 1927) X 10

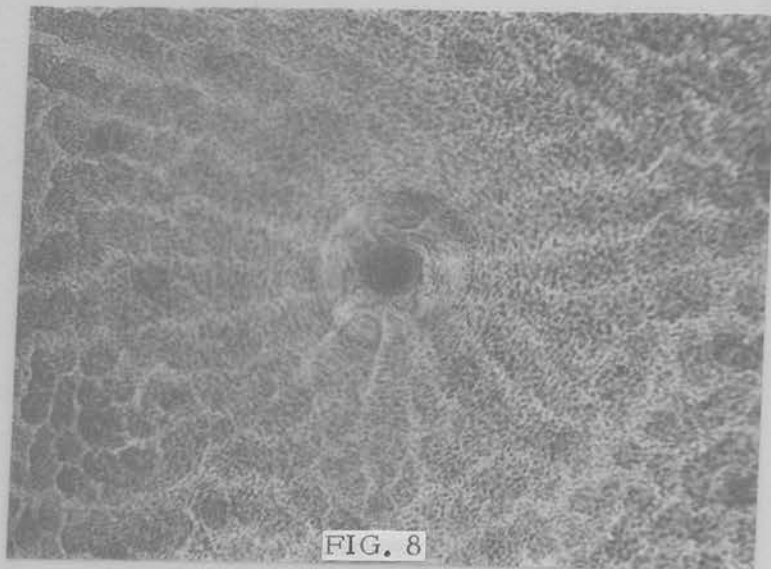


FIG. 8
Whole mount preparation of mammary bud. Male embryo aged 16 days. Haematoxylin. X 120



FIG. 9

Shadowgraph preparation from whole mount of
mammary gland, Female embryo aged 18 days.
X 100

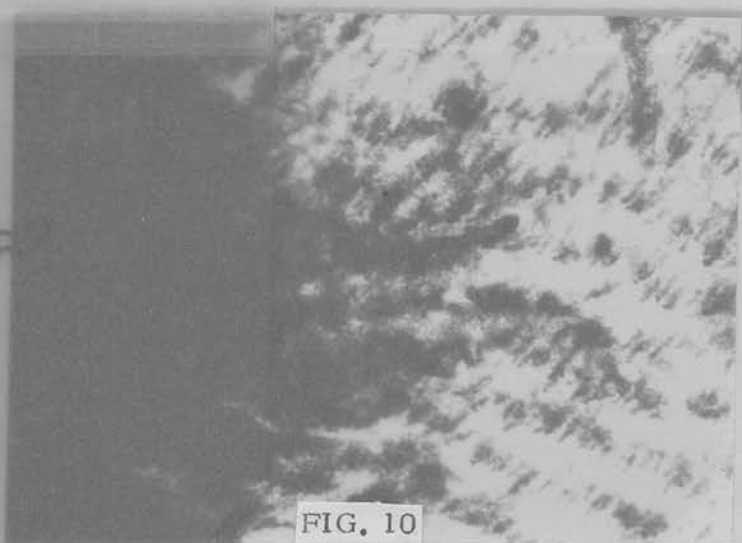


FIG. 10

Fixed pelt of male aged 6 days stained with 1.0%
methylene blue to reveal the position of the
mammary gland.
X 25

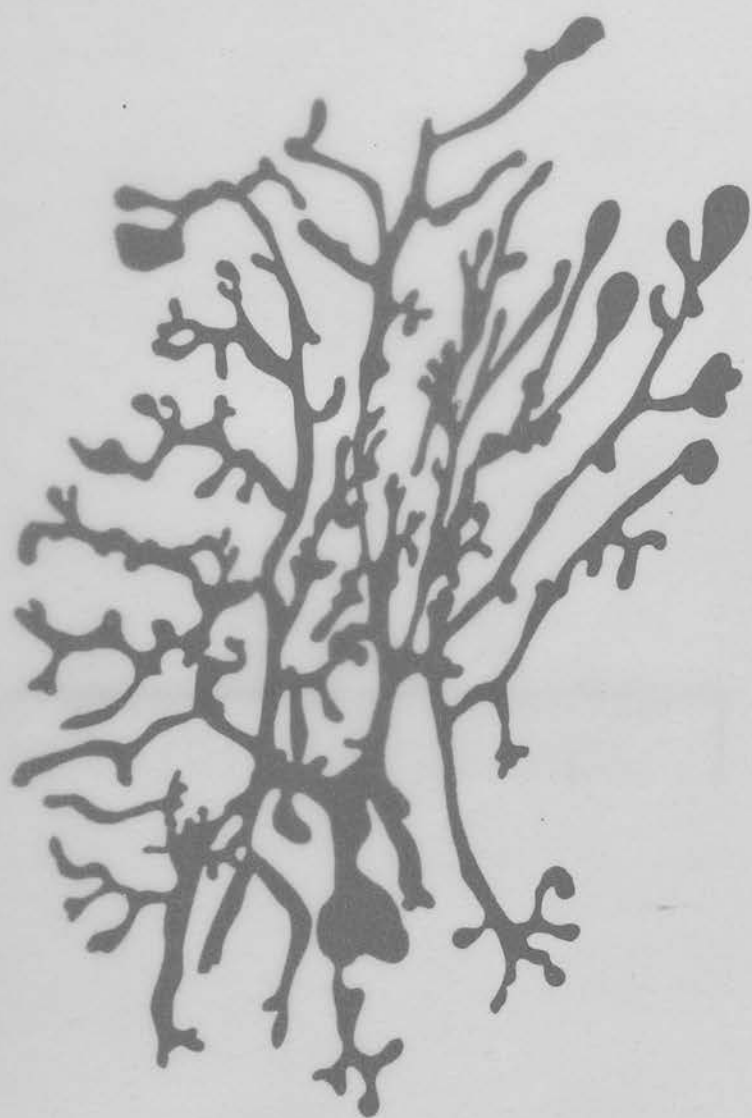


FIG. 11

Shadowgraph preparation from whole mount of mammary gland.
Female in oestrus aged 42 days.

X 25

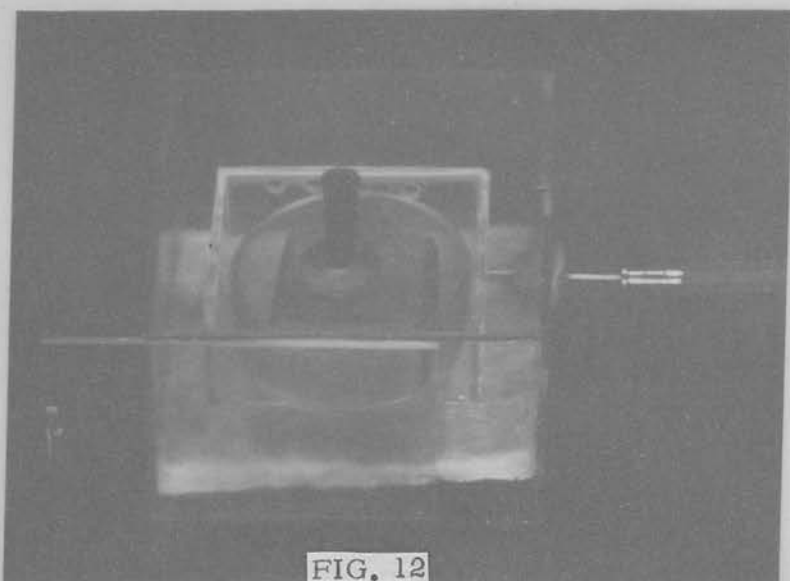


FIG. 12

Photograph of perspex culture chamber showing general features.

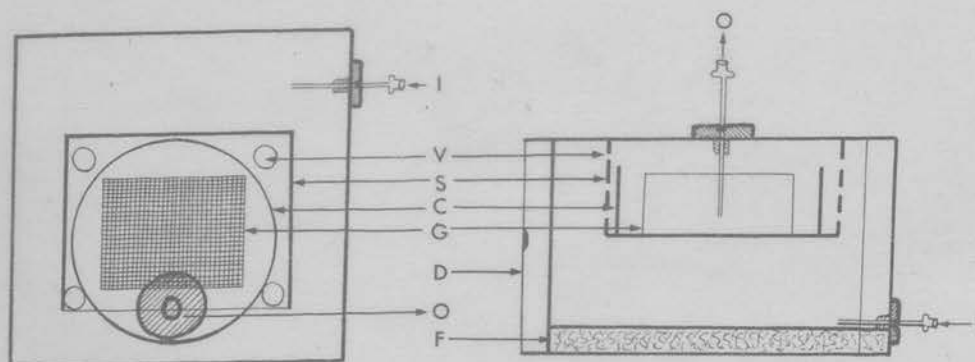


FIG. 13

Scale drawing of culture chamber. On left as seen from above and on right as seen from in front.

- I Gas inlet.
- V Ventilation holes.
- S Shelf for medium container.
- C Glass container for medium.
- G Stainless steel wire grid.
- D Sliding door.
- O Gas outlet.
- F Felt pad.

	Milligrams per 1000 ml.	Approx. equiv. in millimoles
NaCl	6100	104
KCl	450	6
CaCl ₂	220	2
MgSO ₄ ·7H ₂ O	250	1
NaH ₂ PO ₄ ·2H ₂ O	450	3
NaHCO ₃	2820	33.5
Glucose	4000	22
L-Arginine HCl	21	0.1
L-Cysteine HCl	47	0.3
L-Histidine HCl	10	0.05
L-Isoleucine	26	0.2
L-Leucine	26	0.2
L-Lysine HCl	36	0.2
*L-Methionine	7.5	0.05
*L-Phenylalanine	16.5	0.1
*L-Threonine	24	0.2
L-Tryptophan	4	0.02
L-Tyrosine	18	0.1
L-Valine	23	0.2
Thiamine HCl	17	0.05
p-Aminobenzoic acid	35	0.25
Insulin	50	0.001
Chloramphenicol	30	0.1
Phenol red	10	0.03

* Trowell used DL-amino acids at double these concentrations in his original medium.

FIG. 14

Trowells medium T8 (1959). After Paul (1965).

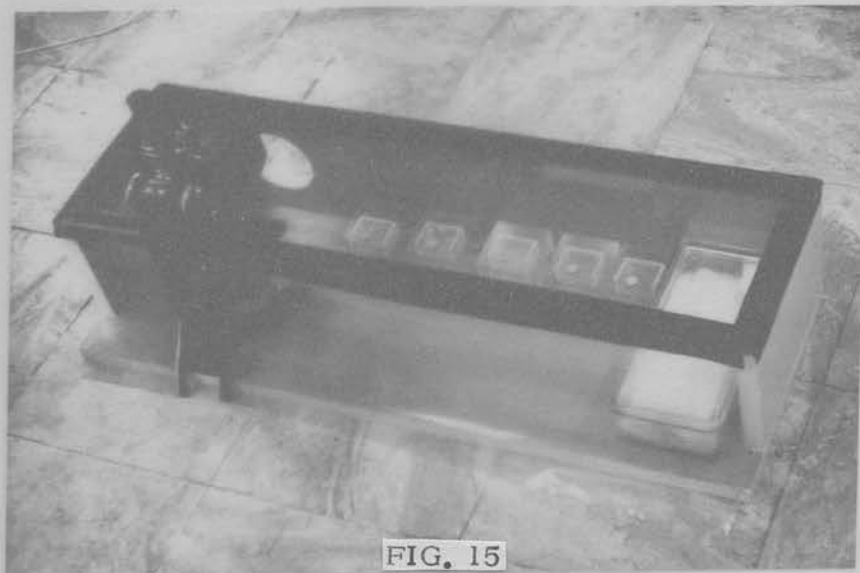


FIG. 15

Hood for prevention of contamination of explants during dissection. After Paul (1965).

1. Embryonic development

1. Embryonic development

During the first 14 days of embryonic development, the embryo and the yolk sac are situated in the uterine wall and are attached to the uterine wall by a placenta. The embryo is situated in the uterine wall and is attached to the uterine wall by a placenta.

(11) 11 days to 14 days. (Hatched stage and formation of primary bud).

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(11) 11 days to 14 days. (Hatched stage and formation of primary bud).

1. Normal development study.

I. Serial sections.

During the 11th to 14 days of prenatal development, the male and female gland rudiments behave identically and will be considered together.

(i) 11 days to 12 days. (Epidermal thickening and formation of primary mammary bud).

The earliest indication of the third pair of thoracic mammary gland rudiments appears on the 11th day of intra-uterine life in both males and females. Gland pairs appear at the same time with no apparent difference in the degree of development on the right and left sides. The first sign is in the form of a localized epidermal thickening at the sites of the future positions of the third pair of thoracic glands. This thickening is produced by an increase in the number of cells of the epidermis (fig. 16), and a lengthening of the epidermal cells involving in particular the basal layer which begins to press downward into the subjacent mesenchyme (fig. 17). This downward projection of the epidermal cells, the primary mammary bud, is separated from the underlying mesenchyme by a clear demarcation line. The mesenchymal cells lying immediately deep to the bud are more numerous than elsewhere, are more closely packed together, and begin to lie with their long axes parallel to the basement membrane of the primary bud. Whereas the primary bud contains only occasional mitotic cells, numerous mitotic figures can be seen in the mesenchyme (fig. 17).

(ii) 13 days to 14 days. (Hillock stage and formation of circular primary bud).

An/

An elevation in the overlying epidermis, the mammary hillock, is now apparent. Growth of the primary bud continues and it becomes rounded off, penetrates deeper into the underlying mesenchyme and eventually is completely circular in shape with only a narrow neck connecting it to the epidermis. The bud consists of an outer ring of cells (similar in appearance to the Malpighian cells of the epidermis) resting on the basement membrane with their long axes pointing towards the centre of the bud which is filled with scattered epithelial cells. A mitosis is rarely seen (fig. 18).

With the bud sinking progressively further into the mesenchyme the mammary hillock disappears, and at the end of the 14th day the superficial surface of the bud is level with the surrounding epidermis (fig. 19).

(iii) 15 days to 16 days. (Formation of the primary sprout)

The spherical bud is now completely imbedded in the mesenchyme and is in contact with the epidermis only by means of a constricted neck at its outer pole. The mammary pit, a slight depression in the epidermis overlying the developing bud now appears. Small blood vessels are seen for the first time in close proximity to the bud and evidence of greater activity can be found in numerous mitotic figures throughout the buds. (figs. 20 and 21).

Male and female buds are still very similar at this stage except for a greater degree of constriction of the neck attachment to the epidermis in the male.

During the 16th day elongation of this connection occurs as/

as the buds begin to form the primary sprout. This primary sprout has a very regular outer ring of stratified columnar cells clearly separated from the mesenchyme which is concentrated in concentric rings around the distal end of the sprout. The central core of the sprout consists of irregularly arranged epithelial cells (figs. 22 and 23).

The greater degree of constriction of the neck attachment of the sprout in the male is the first indication of a clear sexual differentiation. The male and female gland rudiments are therefore considered separately from this stage on.

(iv) (a) Female (17 days).

The primary sprout sinking deeper into the mesenchyme, is now funnel-shaped and turns gradually laterally so that its terminal part lies almost parallel to the skin surface. The distal end of the sprout approaches an opening in the newly formed panniculus carnosus and as it passes through the opening it can occasionally be seen to divide into two secondary sprouts (fig. 24).

(b) Male (17 days).

The primary sprout has become detached from the residue of the original mammary bud and is in the shape of a circular mass of epithelial cells completely surrounded by mesenchymal cells. The gap between the sprout and its bud-residue is filled by cellular connective tissue. There is still a mammary pit present in the overlying epidermis (fig. 25).

(v) (a) Female (18 days).

The primary sprout is much longer and runs parallel to the skin surface laterally. It consists of a basal layer of

low columnar or cuboidal epithelial cells resting on the basement membrane. The inner core of the sprout is filled with irregularly shaped epithelial cells. In most cases the primary sprout divides and forms two secondary sprouts which in turn may give rise to tertiary sprouts deep to the panniculus carnosus. These end in short spherical end-buds which consist of irregular masses of epithelial cells. In some cases similar short end-buds are found appearing along the sides of the primary and secondary sprouts. All branching occurs in one plane parallel to the skin surface.

(b) Male (18 days).

The mammary sprout is completely detached from the epidermis and the mammary pit has disappeared. There is still some condensation of connective tissue between the proximal end of the primary sprout and the overlying epidermis. (fig. 26).

(vi) (a) Female (19 days) (Canalization and early nipple formation).

Further branching of the sprout system has occurred with the number of terminal end-buds approximately doubled in the 24 hours. With lengthening there is also an increase in diameter of portions of the sprouts in conjunction with a tendency of the central core of cells to separate, forming a small cavity or lumen and therefore converting the sprout into a duct. This canalization begins in the distal sprouts first but it also seen in the primary sprout here and there. A short distance from the proximal attachment of the sprout to the epidermis a downgrowth of the deeper layers of the epidermis is seen to occur forming a shallow sulcus in the connective tissue/

tissue which completely surrounds this attachment of the gland.

This is the earliest indication of the nipple formation.

The outer cells of the epidermis overlying the gland rudiment are beginning to form a clearly recognizable stratum corneum with cornification. As a result of this, the mammary pit has disappeared and a rudimentary nipple has formed. The epithelial covering of the rudimentary nipple (the mammary cap), and the epithelial downgrowths surrounding the nipple, together form an epithelial hood over the gland rudiment. (fig. 27).

(b) Male (19 days).

The primary sprout has increased in length and diameter and as in the female begins to show early canalization. More small solid secondary sprouts with end-buds are seen but it is obvious that the male gland is lagging far behind the female in its rate of development.

(vii) (a) Female (20 days).

Further branching with numerous end-buds are seen. Canalization is seen to proceed more proximally towards the nipple but still only appears in short portions of the primary sprout. The epithelial hood is more prominent with the epidermis several layers thicker over the nipple area than in the adjacent skin. The epithelial ingrowth is sinking deeper into the underlying mesenchyme. The anlage of hair follicles are forming in the immediate neighbourhood of the mammary gland.

(b) Male (20 days).

Little change is seen except in overall dimensions and branching with further canalization. The male gland is lagging further behind the female in its branching.

(viii)/

(viii) (a) Female at birth.

The epithelial hood is more prominent. The mammary cap is covered by cornified cells. Some mitotic figures can be seen in the stratum germinativum covering the epithelial projections. The solid column of cells of the original primary sprout has diminished in thickness and is seen passing through the centre of the nipple perpendicular to the skin surface. It is surrounded by a laminated arrangement of connective tissue cells, and almost immediately curves laterally and has a lumen at this point. (figs. 28a and 28b). This, the primary lactiferous duct, is now lined by a stratified cuboidal epithelium of 2 to 3 rows deep which resembles the cells of the stratum germinativum. Passing further laterally the primary duct approaches a vascular lobulated fatty connective tissue deposit which lies deep to the dermis and panniculus carnosus. As soon as it reaches the fatty connective tissue it begins to branch and at the same time passes through an opening in the panniculus. Secondary and tertiary branches appear, displaying predominantly a simple or pseudostratified cuboidal epithelium, but in several places the epithelium is still clumped in several layers with the duct lumen not completely patent. Epithelial debris can also be seen in some ducts. (fig. 29). All ducts end in a short solid end-bud of epithelial cells of about 15 to 20 microns in length. Occasionally mitotic figures can be seen in these cells. The branching system of ducts and end-buds distributes itself deep to the panniculus carnosus in a flat sheet parallel to the skin surface. Immediately/

Immediately surrounding all segments of the ducts are concentrically arranged layers of connective tissue.

Immediately deep to the epithelial hood the connective tissue is dense, and is arranged parallel to the stratum germinativum near the walls of the hood. Further away the connective tissue cells are more irregularly arranged until they form the concentric layers around the primary duct.

(b) Male at birth.

The male gland has changed very little. There is some increase in the number of end-buds, and canalization extends almost along the entire length of the primary duct.

(ix) (a) Female (24 hours postnatal).

No significant changes from the appearance at birth are observed.

The primary lactiferous duct shows a lumen earlier in its course through the nipple but still does not open out on the nipple surface (figs. 30a and 30b). The secondary and tertiary branches are better canalized and begin to display a simple cuboidal epithelium. Some cell debris, however, can still be seen in the ducts. The panniculus carnosus has undergone considerable development (fig. 31).

(b) Male (24 hours postnatal)

No changes are observed.

(x) (a) Female (4 to 6 days).

During this period canalization is completed and the primary lactiferous duct is patent, opening to the surface through the milk pore near the summit of the nipple. The nipple is no more elevated than before but the downward projections/

projections of the epithelial hood extend more deeply into the mesenchyme (fig. 32). The lumen of the intra-epidermal part of the primary duct is continuous with that of the intra-dermal part of the duct which curves laterally immediately after passing through the nipple to run almost parallel to the skin surface. Its lumen is very distinct and is again lined by a stratified cuboidal epithelium of two to three layers thick (fig. 33). A few normal nucleated cells resembling the cells of the epithelial wall can be seen in the lumen of the primary duct and its secondary branches (fig. 34). Some smooth muscle fibres lying in the deep part of the dermis can be seen passing deep to the epithelial hood and running obliquely towards the surface appear to enter into the formation of the periductal connective tissue (figs. 32 and 33). Some of these fibres run upwards to the inner surfaces of the epithelial downgrowths. The primary duct branches and passes through the panniculus as before and spreads out in the typical uniplanar distribution of the duct system immediately deep to the panniculus (figs. 34 and 35). Secondary and tertiary branches are lined by a simple cuboidal epithelium in all cases. Towards the periphery of the gland myo-epithelial cell nuclei can be seen lying between the epithelium and its basement membrane (fig. 36). The lumen of the duct system stops from 15 to 20 microns short of the tip of the end-buds which consist of a mass of irregularly arranged epithelial cells. The gland stroma is richly vascular in the periductal regions. (fig. 37).

(b)/

(b) Male (4 to 6 days).

The male gland is still detached from the overlying skin. As in the female, canalization is completed during this period. The duct system is surrounded by a well formed layer of connective tissue lying inside the sub-pannicular areolar tissue (fig. 38). There is no difference in the epithelial lining of the original primary duct and its side branches. The lining varies from a simple cuboidal to a pseudostratified cuboidal type of epithelium occasionally displaying large swollen multinucleated cells with pale staining nuclei. The lumen is patent in the whole duct system except in the end-bud regions which like those of the female gland consists of a mass of epithelial cells. The periductal region is richly supplied with blood vessels (fig. 39).

(xi) (a) Female (8 to 21 days).

No change in the histology is observed during this period, the glands retaining all the features of the 6 day stage.

(b) Male (8 to 21 days).

The gland remains rudimentary in its structure with no change in its histology.

II. Whole mounts.

(i) Quantitative analysis of the measurements obtained from whole mount preparations.

A typical whole mount preparation with its shadowgraph is shown in fig. 40. Serial whole mount shadowgraphs, randomly selected from male and female mice over the age of 18 days prenatal to 42 days postnatal are shown in fig. 41 to illustrate the overall changes in the general shape and structure of the mammary tree. The shadowgraph preparations with their measurements/

measurements represented in this series are shown in figs. 42 to 63. No measurements were carried out on the 42 day old male and female shadowgraph preparations. Composite tables of all the measurements relating to the prenatal and postnatal development of the glands in male and female mice are shown in Tables 2 to 5. These tables summarise changes in body weight, number of end-buds, gland outline surface area and total duct length. The following observations are made:-

(a) Body weight. (Graphs 1 and 2).

Throughout development the body weight in both males and females increases in almost exactly similar fashion over the ages studied. Therefore all measurements are related to age and body weight.

(b) Number of end-buds. (Graphs 3, 4, 5 and 6).

Female glands.

The number of end-buds related both to age and body weight increases very rapidly during the prenatal period and reaches a mean maximum of 48 at approximately 4 days after birth or at a mean body weight of 1.78 grams. It then tends to stabilise in both relations to a number between 40 and 50.

Male glands.

The number of end-buds related both to age and body weight also increases during the prenatal period but reaches a mean maximum of only 17 end-buds at approximately 24 hours after birth or at a mean body weight of 1.43 grams. It then gradually diminishes in number to reach a mean value of 10 end-buds at 21 days of age or at a mean body weight of 6.88 grams.

(c) Total duct length (Graphs 7, 8, 9 and 10).

Female/

Female glands.

The total duct length related to age and mean body weight respectively shows a steep rise in length which is most marked during the prenatal phase but is continued in the postnatal period to reach a mean maximum of 13.27% at 21 days of age or at a mean body weight of 7.17 grams.

Male glands.

The total duct length related to age and mean body weight respectively shows a rise which is much more gradual than in the female, most marked during the prenatal period but continued in the postnatal period to reach a mean level of 2.64% at 8 days of age or at a mean body weight of 3.21 grams. Thereafter the duct length fluctuates between 1.96% and 2.76% in length.

(d) Outline surface area (Graph 11).

Female glands.

The mean outline surface area related to age shows a steady rise which is maintained throughout the prenatal and postnatal development to reach a maximum value of 0.768 sq.% at 21 days of age.

Male glands.

The mean outline surface area related to age shows a much slower rise than that of the female gland. This rise is maintained throughout prenatal development to reach a value of 0.063 sq.% at 24 hours postnatal age after which it fluctuates between 0.039 sq.% and 0.108 sq.%.

(ii) Relative growth analysis.

All measurements relating to the relative growth analysis of the male and female glands during prenatal and postnatal development/

development are given in Table 6. The body weight measurements represent the body weight of each animal studied. The gland area measurements represent the mean outline gland area of paired glands in each animal studied.

(a) Prenatal.

Female glands (Graph 12)

Over the body weight range of 712.0 mgm to 1175.0 mgm ($\log(w)^{\frac{2}{3}} = 1.9018$ to 2.0467) the slope of the regression line = 3.2.

Male glands (Graph 12)

Over the body weight range of 700.0 mgm to 1156.0 mgm ($\log(w)^{\frac{2}{3}} = 1.8964$ to 2.0420) the slope of the regression line = 4.2.

(b) Postnatal.

Female glands (Graph 13)

Over the body weight range of 1.37 gm to 7.80 gm ($\log(w)^{\frac{2}{3}} = 0.0911$ to 0.5947) the slope of the regression line = 0.86.

Male glands (Graph 13)

Over the body weight range of 1.35 gm to 7.45 gm ($\log(w)^{\frac{2}{3}} = 0.0868$ to 0.5814) the slope of the regression line = 0.55.

(iii) Comparison of left and right paired glands.

The number of end-buds in the left and right third thoracic mammary gland pairs of 33 males and 33 female mice are compared in Table 7 and analysed in Histograms 1 to 5.

(a) Females (Table 7 and Histograms 2 and 3)

The total number of end-buds on the right is 1350

(arithmetic/

(arithmetic mean = 40.90 per gland) with a range of 5.1 and on the left is 1346 (arithmetic mean = 40.78 per gland) with a range of 4.78. The modal value is 41 and occurs in 13 glands (6 left and 7 right). 6 pairs of glands have the same number of end-buds and the difference in number of end-buds between paired glands never exceed 7.

(b) Males (Table 7 and Histograms 1 and 3)

The total number of end-buds on the right is 346 (arithmetic mean = 10.48 per gland) with a range of 3.52 and on the left is 343 (arithmetic mean = 10.40 per gland) with a range of 3.60. The modal value is 11 and occurs in 17 glands (9 left and 8 right). 7 pairs of glands have the same number of end-buds and the difference in number of end-buds between paired glands never exceed 3.

The distribution of the total number of end-buds in male and female glands are shown in Histograms 4 and 5.

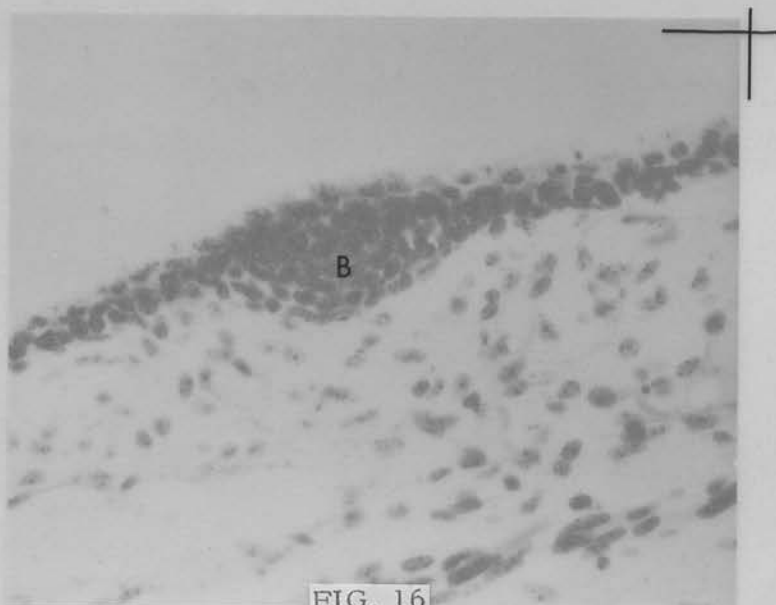


FIG. 16

Transverse section through the mammary rudiment
in an eleven-day male embryo.

Peters.

X 330

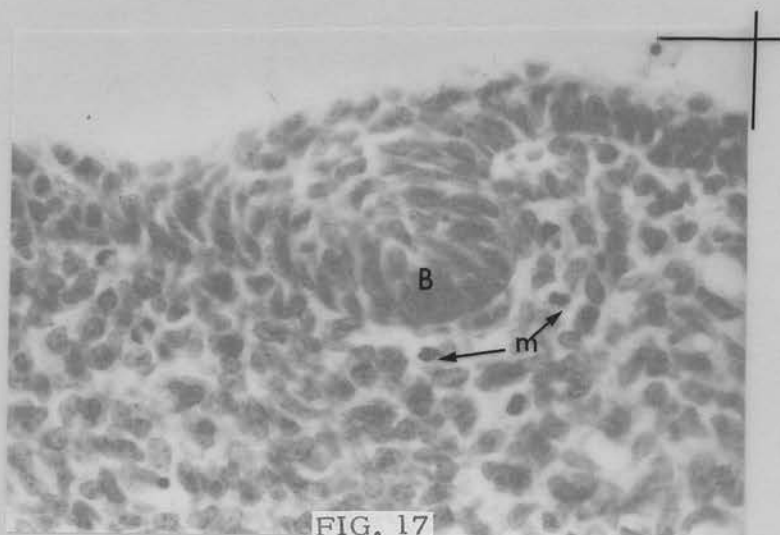


FIG. 17

Transverse section through the mammary rudiment
in a twelve-day female embryo.

Haematoxylin and eosin.

X 528

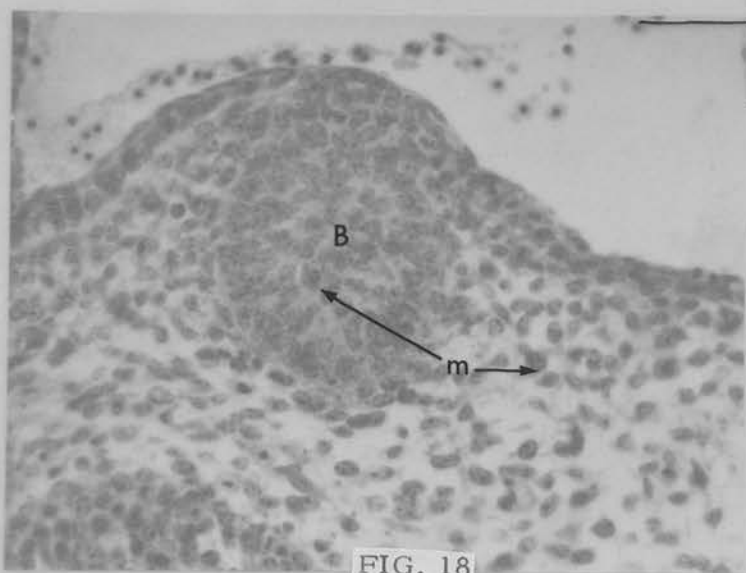


FIG. 18

Transverse section through the mammary bud
in a thirteen-day male embryo.

Haematoxylin and eosin. X 400

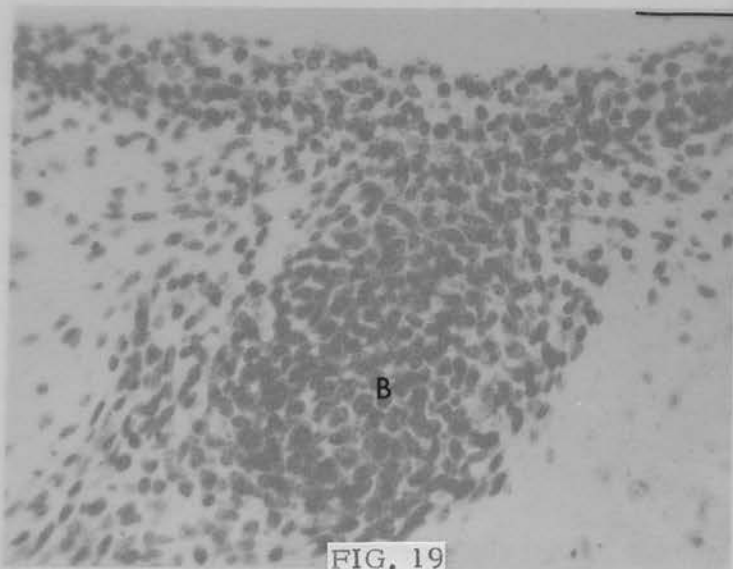


FIG. 19

Transverse section through the mammary bud
in a fourteen-day female embryo.

Peters. X 297



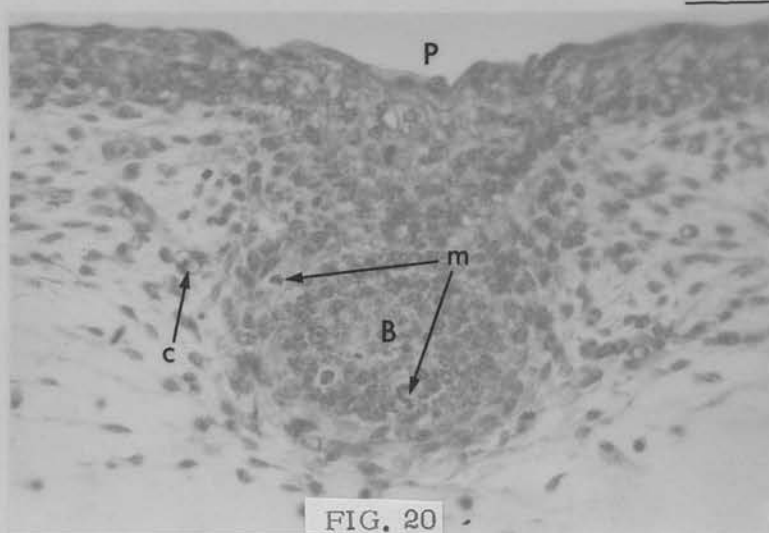


FIG. 20

Transverse section through the mammary bud
in a fifteen-day male embryo.

Haematoxylin and eosin. X 330.

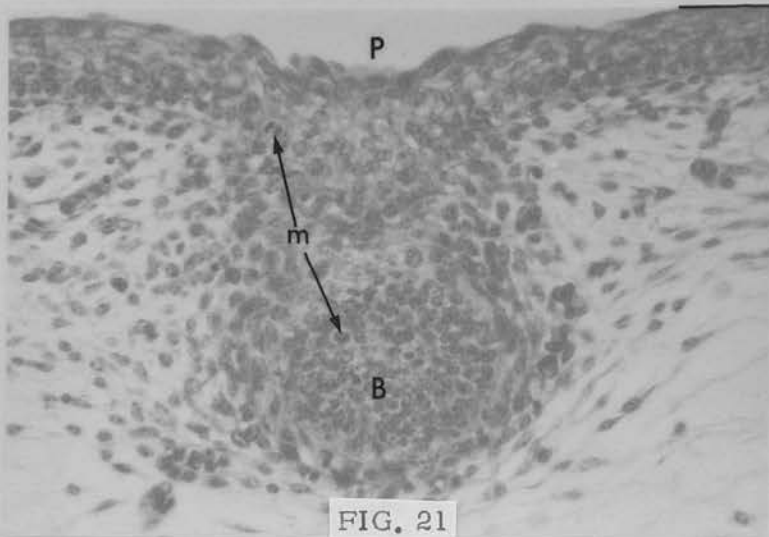


FIG. 21

Transverse section through the mammary bud
in a fifteen day female embryo.

Haematoxylin and eosin. X 330.

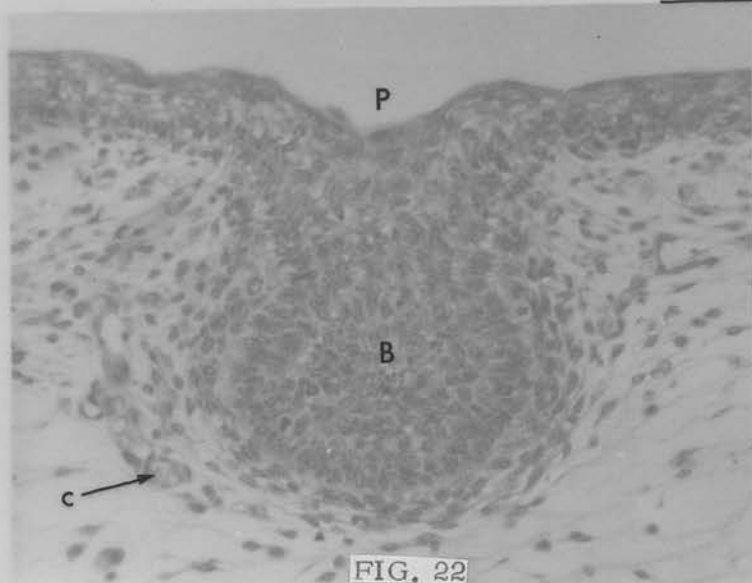


FIG. 22

Transverse section through the mammary bud
in a sixteen-day female embryo.

Mallory. X 330

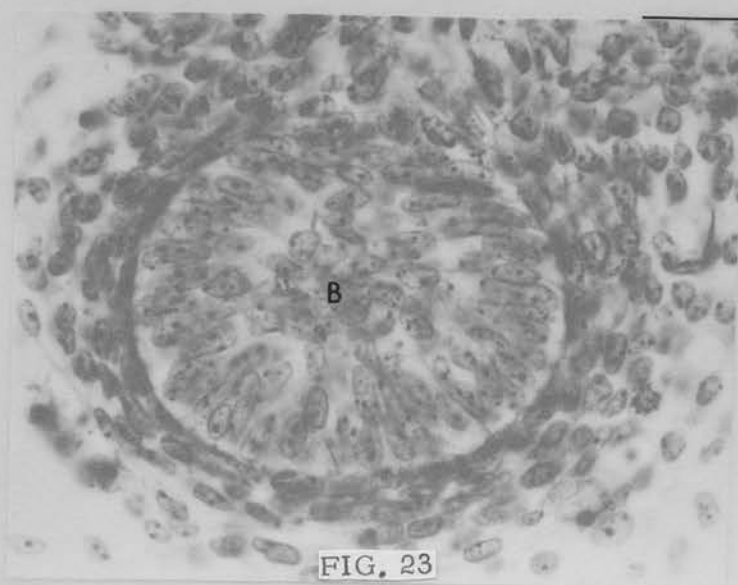


FIG. 23

Transverse section through the mammary bud
in a sixteen-day female embryo.

Peters. X 528

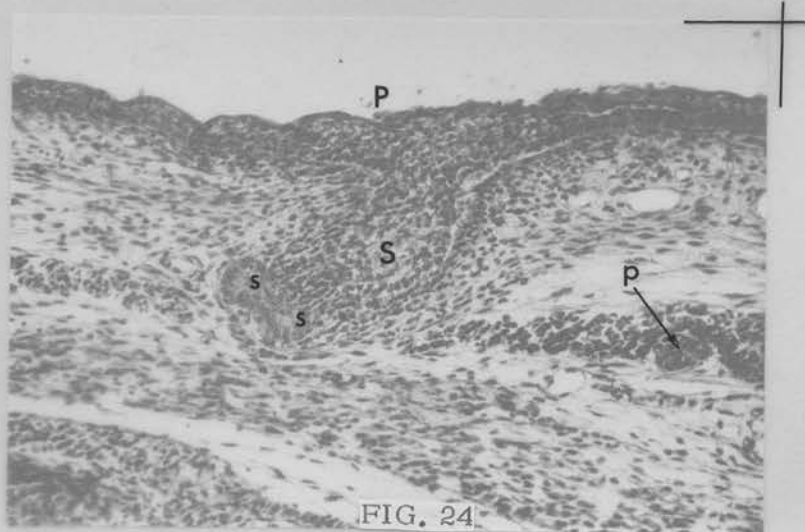


FIG. 24

Transverse section through the mammary sprout
in a seventeen-day female embryo.

Haematoxylin and eosin. X 120

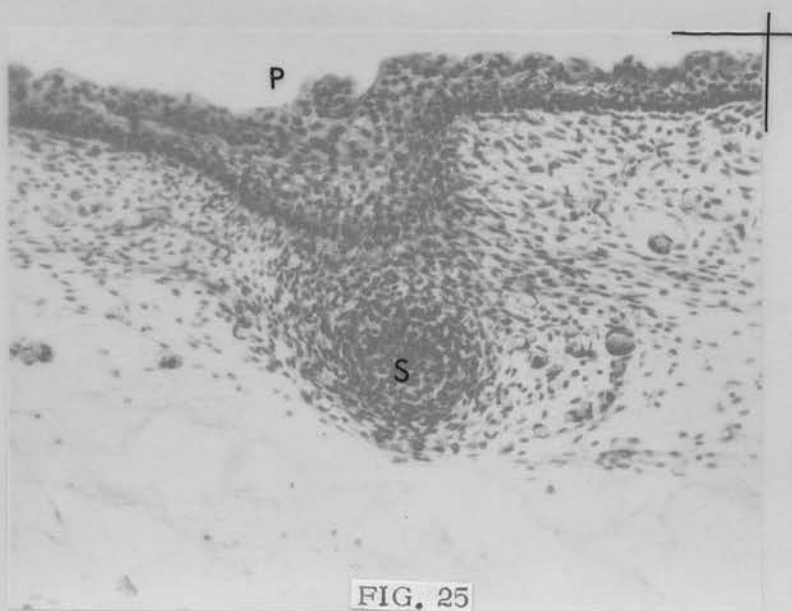


FIG. 25

Transverse section through the mammary sprout
in a seventeen-day male embryo.

Haematoxylin and eosin. X 120

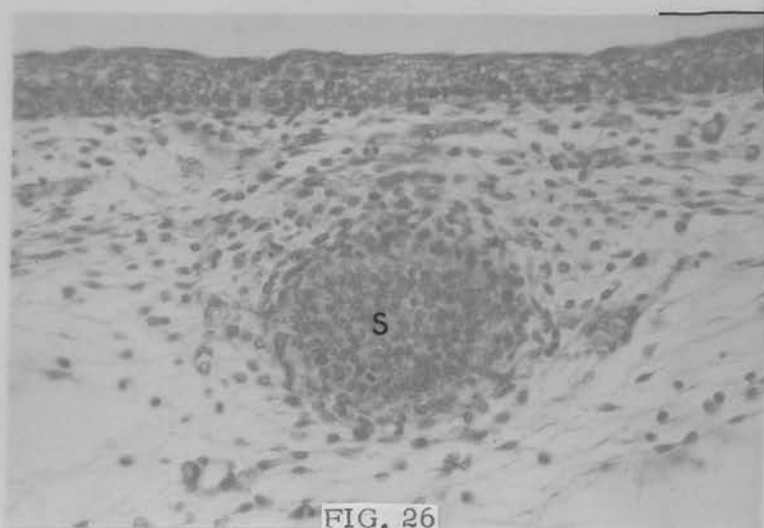


FIG. 26

Transverse section through the mammary sprout
in an eighteen-day male embryo.

Haematoxylin and eosin.

X 277

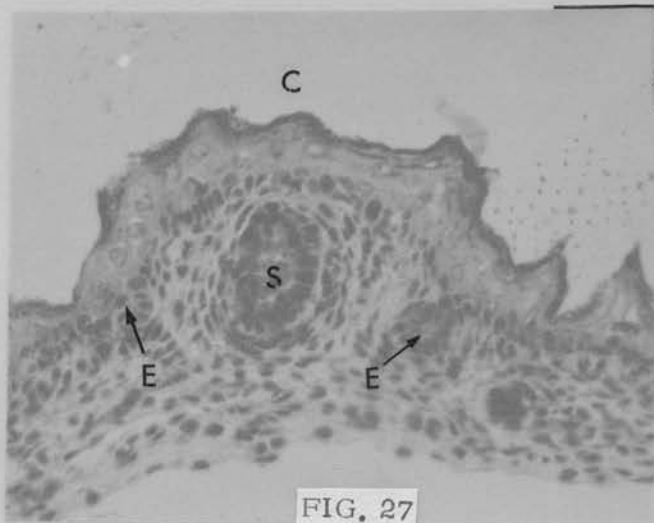


FIG. 27

Transverse section through the rudimentary
nipple in a nineteen-day female embryo.

Haematoxylin and eosin.

X 330

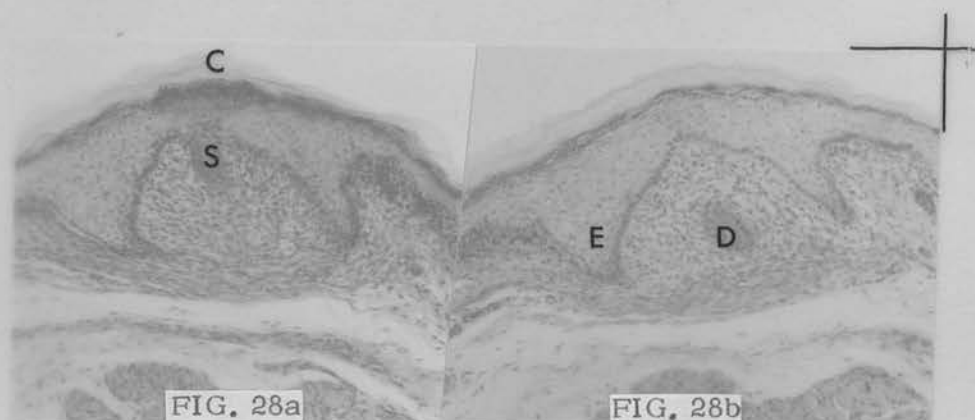


FIG. 28a
FIG. 28b
Transverse section through the nipple. Female
at birth.

Haematoxylin and eosin. X 132

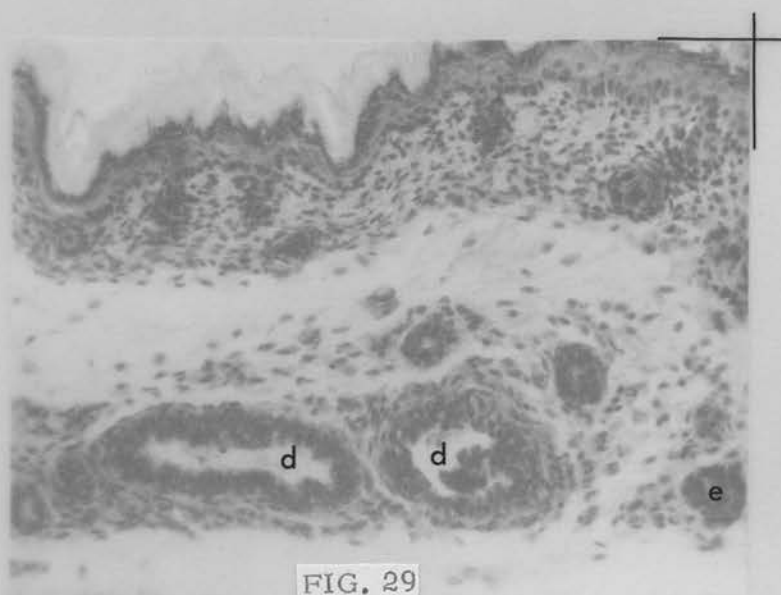
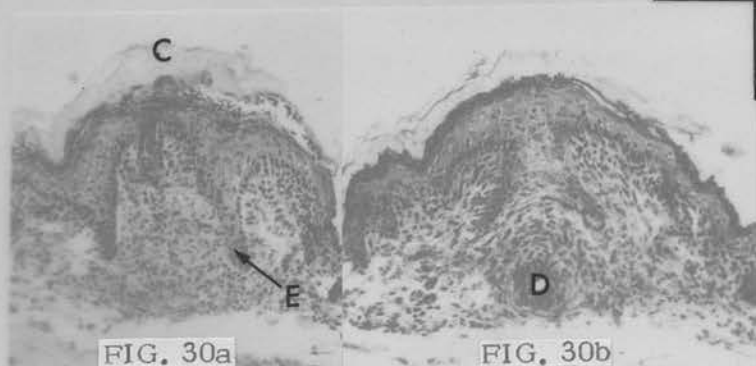


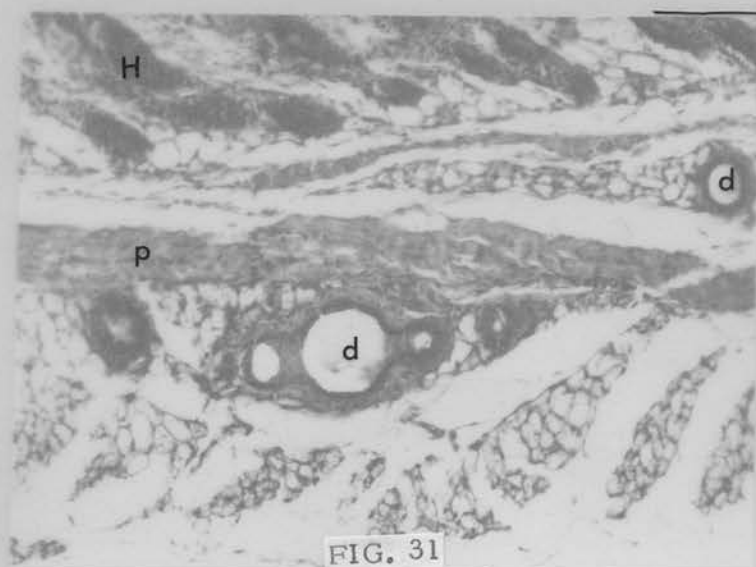
FIG. 29
Transverse section through the branching duct-
system. Female at birth.

Haematoxylin and eosin. X 250



Transverse sections through the nipple, Female aged 24 hours.

Haematoxylin and eosin. X 132



Transverse section through the branching duct-system, Female aged 24 hours.

Haematoxylin and eosin. X 132

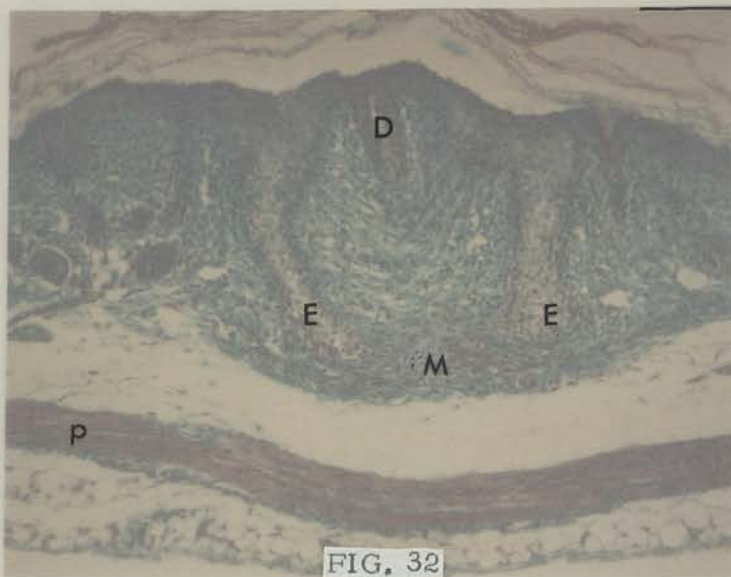


FIG. 32

Transverse section through the nipple. Female aged 6 days.

Masson. X 132

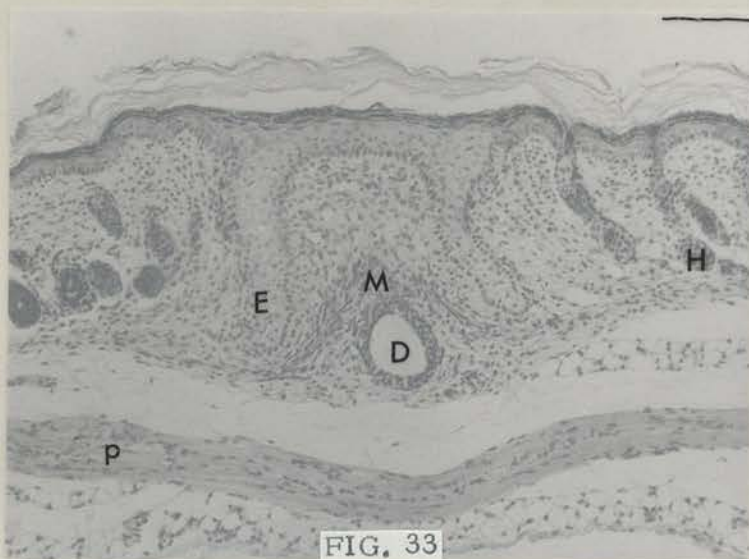


FIG. 33

Transverse section through the primary duct. Female aged 6 days.

Haematoxylin and eosin. X 132



FIG. 34

Transverse section of primary duct and first secondary branch about to pass through the opening in the panniculus carnosus. Female aged 6 days.

Haematoxylin and eosin. X 132

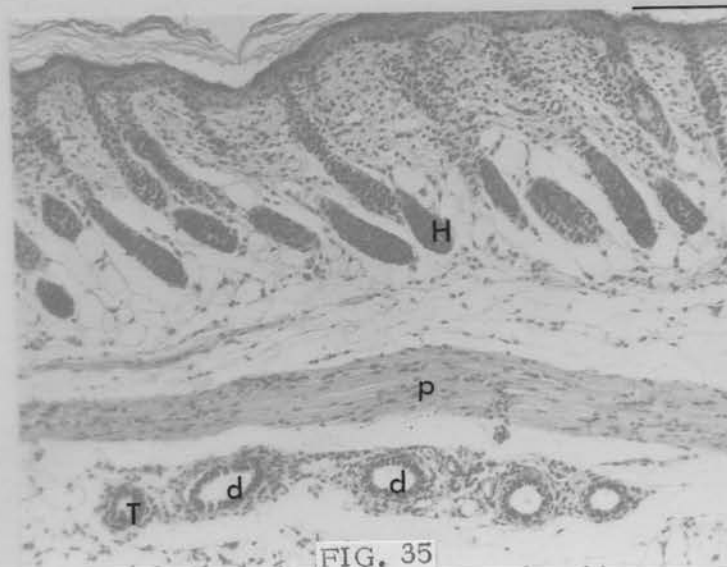


FIG. 35

Transverse section illustrating the uniplanar distribution of the secondary duct-system. Female aged 6 days.

Haematoxylin and eosin. X 132

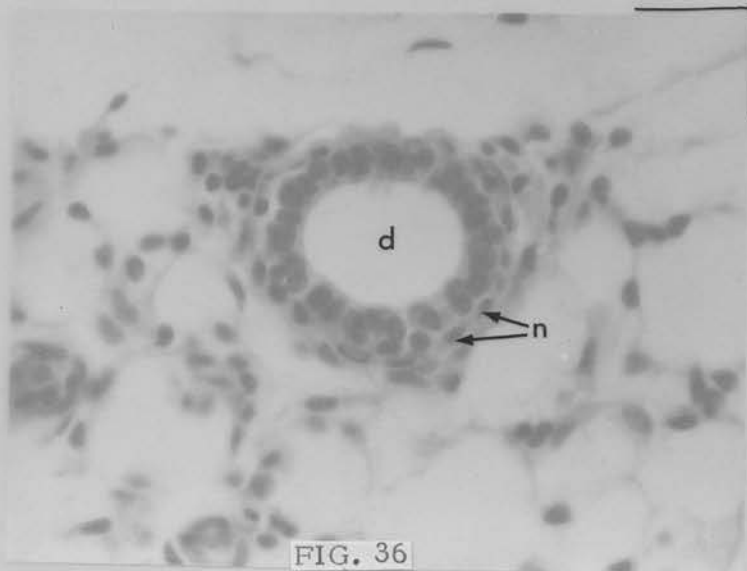


FIG. 36

Transverse section through a secondary duct.
Female aged 6 days.

Masson. X 330

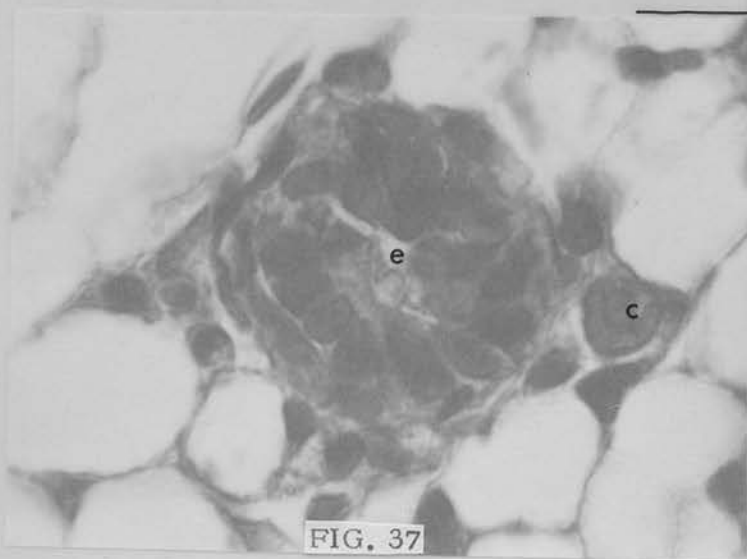
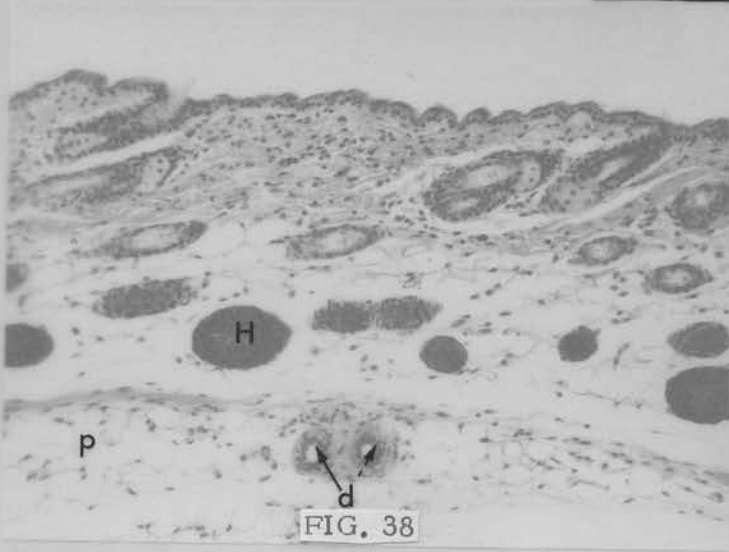


FIG. 37

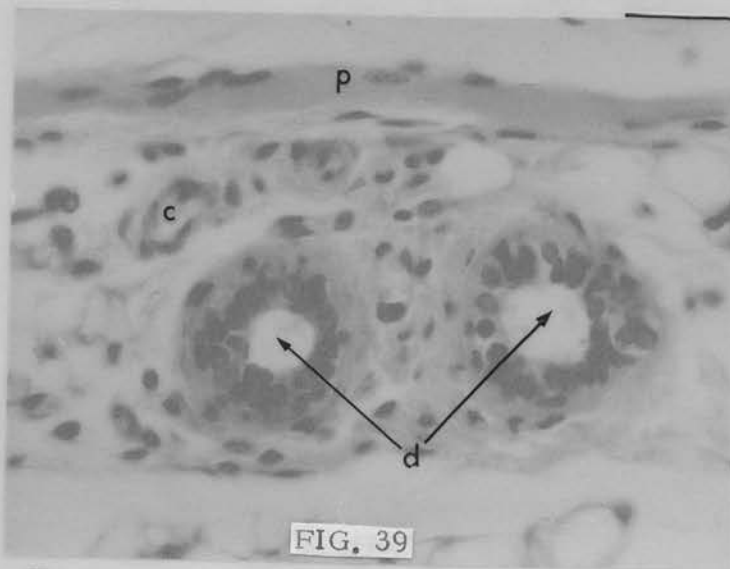
Transverse section through the terminal part
of an end-bud. Female aged 6 days.

Masson. X 1320



Transverse section through the duct-system,
Male aged 6 days.

Masson, X 132



Transverse section through the duct-system,
Male aged 6 days.

Masson, X 330



FIG. 40

Whole mount and shadowgraph preparation of mammary gland. Male aged 4 days.

Haematoxylin. X 128

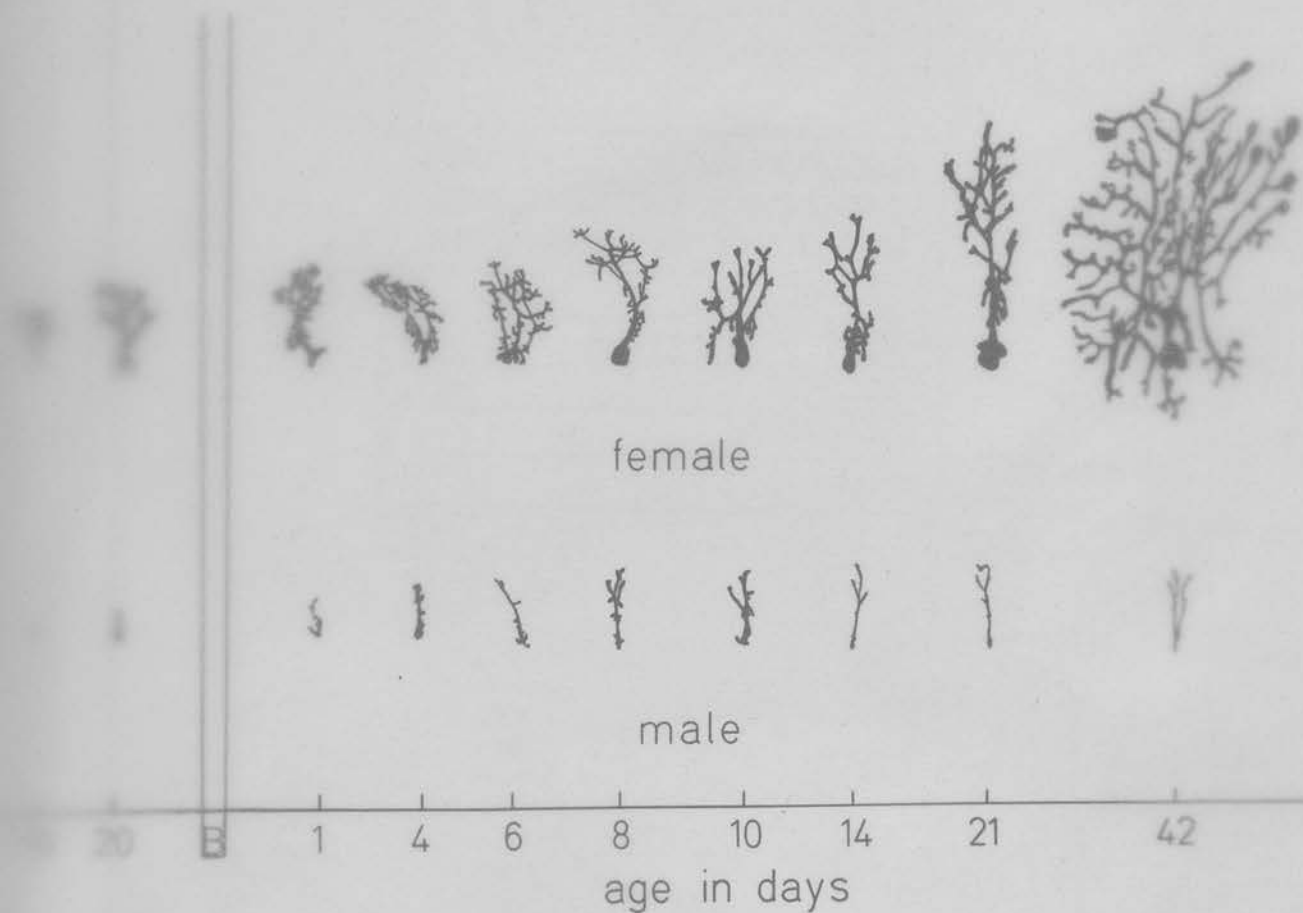


FIG. 41

Serial whole mount shadowgraphs of mammary glands from male and female mice, illustrating the changes in the general outline of the mammary tree during late prenatal and early postnatal development.

X 6,25



FIG. 42

Whole mount shadowgraph of mammary gland.
 Prenatal male aged 18 days. X 100.
 Total number of end-buds 3.
 Total duct length 0.32mm.
 Gland area 0.011sq.mm.
 Body weight 740mgm.



FIG. 43

Whole mount shadowgraph of mammary gland.
 Prenatal female aged 18 days. X 100.
 Total number of end-buds 15.
 Total duct length 2.10mm.
 Gland area 0.074sq.mm.
 Body weight 760mgm.



FIG. 44

Whole mount shadowgraph of mammary gland.
 Prenatal male aged 19 days. X 100.
 Total number of end-buds 5.
 Total duct length 0.625mm.
 Gland area 0.020sq.mm.
 Body weight 875mgm.

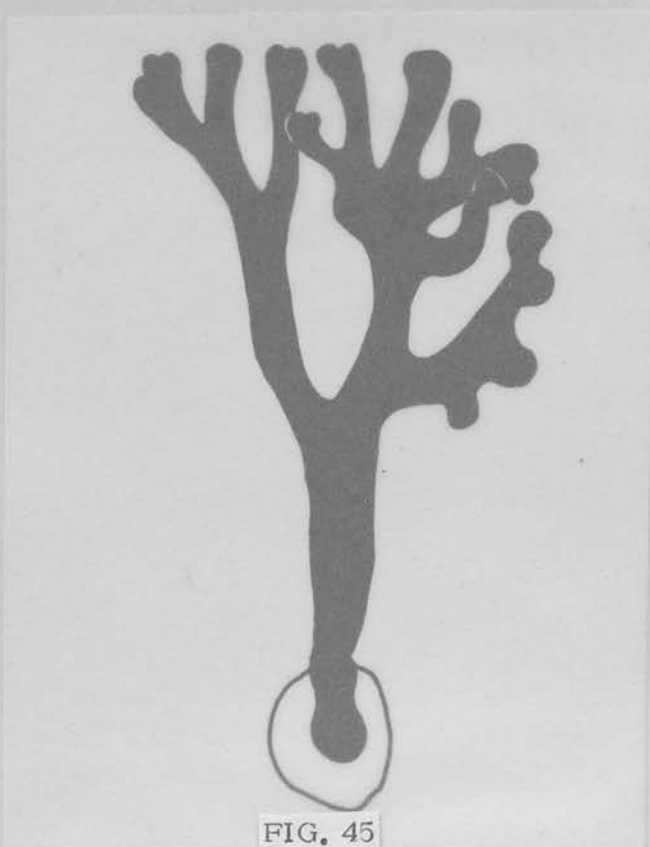


FIG. 45

Whole mount shadowgraph of mammary gland.
 Prenatal female aged 19 days. X 100.
 Total number of end-buds 19.
 Total duct length 3.80mm.
 Gland area 0.088sq.mm.
 Body weight 900mgm.



FIG. 46

Whole mount shadowgraph of mammary gland,
 Prenatal male aged 20 days, X 50.
 Total number of end-buds 8.
 Total duct length 0.910mm.
 Gland area 0.042sqmm.
 Body weight 1040mgm.

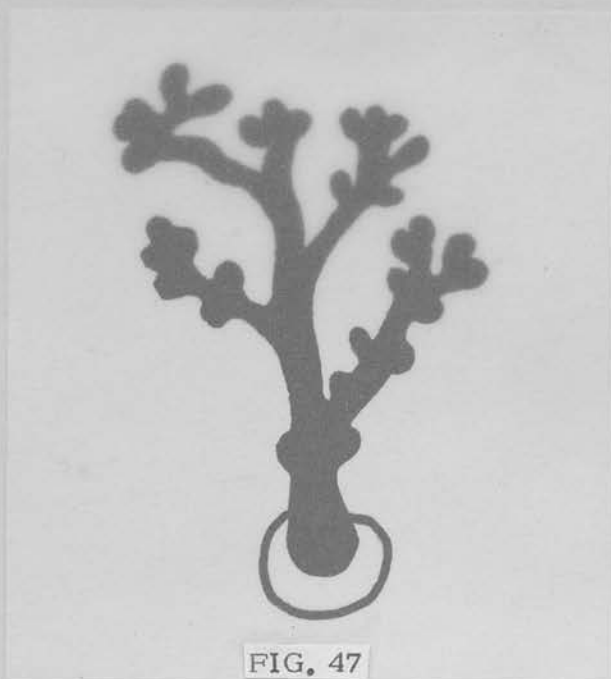


FIG. 47

Whole mount shadowgraph of mammary gland,
 Prenatal female aged 20 days, X 50.
 Total number of end-buds 31.
 Total duct length 6.000 mm.
 Gland area 0.118sq.mm.
 Body weight 1055mgm.



FIG. 48

Whole mount shadowgraph of mammary gland.
 Male aged 24 hours. X 25
 Total number of end-buds 8.
 Total duct length 1.12mm.
 Gland area 0.038sq.mm.
 Body weight 1.35g.



FIG. 49

Whole mount shadowgraph of mammary gland.
 Female aged 24 hours. X 25
 Total number of end-buds 48.
 Total duct length 9.20mm.
 Gland area 0.263sq.mm.
 Body weight 1.37g.



FIG. 50

Whole mount shadowgraph of mammary gland.
 Male aged 4 days. X 25
 Total number of end-buds 15.
 Total duct length 1.36mm.
 Gland area 0.076sq.mm.
 Body weight 1.65g.



FIG. 51

Whole mount shadowgraph of mammary gland.
 Female aged 4 days. X 25
 Total number of end-buds 57.
 Total duct length 10.5mm.
 Gland area 0.372sq.mm.
 Body weight 1.80g.



FIG. 52

Whole mount shadowgraph of mammary gland.

Male aged 6 days.

X 25

Total number of end-buds 10.

Total duct length 1.87mm.

Gland area 0.070sq.mm.

Body weight 2.80g.

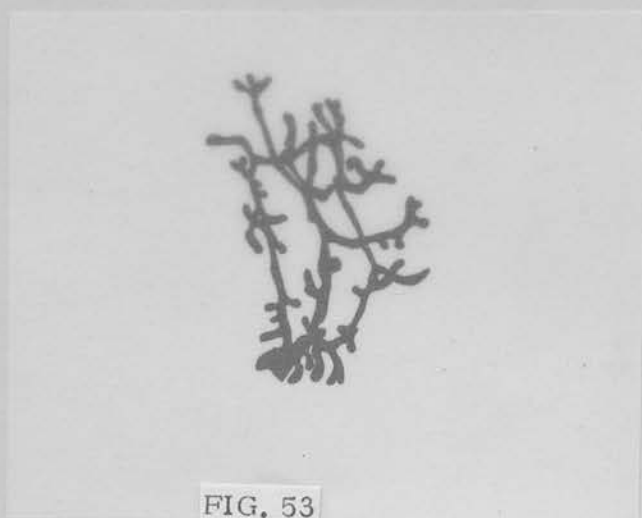


FIG. 53

Whole mount shadowgraph of mammary gland.

Female aged 6 days.

X 25

Total number of end-buds 60.

Total duct length 11.10mm.

Gland area 0.429sq.mm.

Body weight 3.10g.

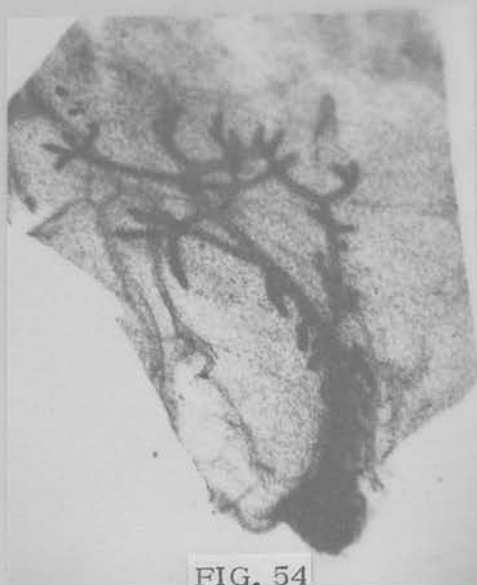


FIG. 54

Whole mount of mammary gland. Female aged 8 days.

Haematoxylin. X 25



FIG. 55

Shadowgraph of whole mount. (FIG. 54) X 25

Total number of end-buds 51.

Total duct length 11.00mm.

Gland area 0.424sq.mm.

Body weight 3.25g.



FIG. 56

Whole mount of mammary gland, Male aged
8 days.

Haematoxylin. X 25



FIG. 57

Shadowgraph of whole mount. (FIG. 56) X 25

Total number of end-buds 18.

Total duct length 3.92mm.

Gland area 0.120sq.mm.

Body weight 2.65g.



FIG. 58

Whole mount shadowgraph of mammary gland.
 Male aged 10 days. X 25
 Total number of end-buds 12.
 Total duct length 1.40mm.
 Gland area 0.069sq.mm.
 Body weight 4.40g.

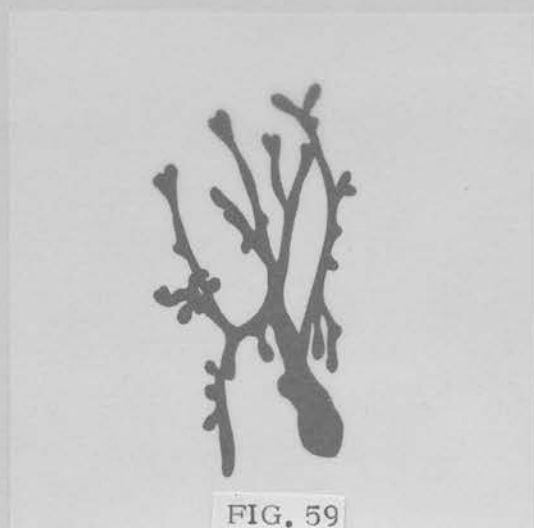


FIG. 59

Whole mount shadowgraph of mammary gland.
 Female aged 10 days. X 25
 Total number of end-buds 36.
 Total duct length 10.60mm.
 Gland area 0.510sq.mm.
 Body weight 4.00g.



FIG. 60

Whole mount shadowgraph of mammary gland.
 Male aged 14 days. X 25
 Total number of end-buds 10.
 Total duct length 2.30mm.
 Gland area 0.810sq.mm.
 Body weight 6.55g.



FIG. 61

Whole mount shadowgraph of mammary gland.
 Female aged 14 days. X 25
 Total number of end-buds 36.
 Total duct length 9.60mm.
 Gland area 0.415sq.mm.
 Body weight 5.25g.



FIG. 62

Whole mount shadowgraph of mammary gland.
 Male aged 21 days. X 25
 Total number of end-buds 12.
 Total duct length 1.91mm.
 Gland area 0.069sq.mm.
 Body weight 6.60g.

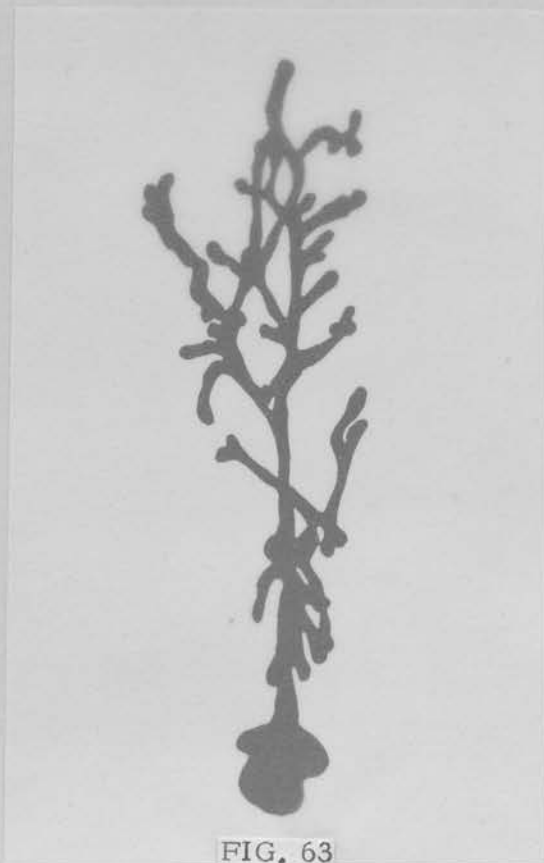


FIG. 63

Whole mount shadowgraph of mammary gland.
 Female aged 21 days. X 25
 Total number of end-buds 41.
 Total duct length 13.90mm.
 Gland area 0.795sq.mm.
 Body weight 7.50g.

AGE IN DAYS.	NUMBER OF EMBRYOS.	WEIGHT IN MGM. (max) mean (min)	NUMBER OF GLANDS.	NUMBER OF END-BUDS. (max) mean (min)	AREA IN SQ.MM. (max) mean (min)	DUCT-LENGTH IN MM. (max) mean (min)
11	5	(20,00) 19,50	(epidermal thickening) 10	-	-	-
12	5	(18,50) (53,50) 52,00	(primary bud hillock stage) 9*	-	-	-
14	4	(50,00) (144,5) 140,0	(primary bud circular stage) 8	-	-	-
16	5	(136,5) (437,5) 420,0	(primary sprout) 10	1	-	-
17	5	(410,5) (561,0) 540,5	10	1	-	-
18	6	(525,0) (785,0) 750,0	12	(16) 11	(0,093) 0,051	(2,950) 2,050
19	5	(712,5) (935,5) 890,0	10	(7) (34) 24	(0,045) (0,120) 0,095	(1,900) (4,800) 4,250
20	6	(875,0) (1175,0) 1080	12	(18) (41) 33	(0,087) (0,143) 0,129	(3,760) (7,120) 6,070
		(1005,0)		(27)	(0,105)	(5,910)

TABLE 2

COMPOSITE TABLE OF MEASUREMENTS RELATING TO THE PRE-NATAL DEVELOPMENT OF THE MAMMARY GLAND IN FEMALE MICE.

*In one of the 12 day old embryos the right gland rudiment was absent.

AGE IN DAYS.	NUMBER OF EMBRYOS.	WEIGHT IN MGM. (max) mean (min)	NUMBER OF GLANDS.	NUMBER OF END-BUDS. (max) mean (min)	AREA IN SQ.MM. (max) mean (min)	DUCT-LENGTH IN MM. (max) mean (min)
10	5	(20,50) 19,00	(epidermal thickening) 10	-	-	-
12	4	(18,00) (51,50) 48,00	(primary bud hillock stage) 8	-	-	-
14	5	(46,00) (143,5) 135,5	(primary bud circular stage) 10	-	-	-
16	5	(129,0) (423,0) 410,5	(primary sprout) 10	1	-	-
18	5	(401,5) (538,5) 528,0	10	1	-	-
20	5	(516,0) (768,5) 748,0	10	(4) 3	(0,019) 0,012	(0,420) 0,350
22	5	(731,0) (925,0) 887,0	10	(2) (8) 6	(0,009) (0,031) 0,025	(0,205) (0,650) 0,630
24	5	(868,5) (1156,0) 1076	10	(3) (13) 10	(0,018) (0,055) 0,046	(0,615) (1,206) 0,945
26	5	(994,0)	10	(7)	(0,032)	(0,840)

TABLE 3

COMPOSITE TABLE OF MEASUREMENTS RELATING TO THE PRE-NATAL DEVELOPMENT OF THE MAMMARY GLAND IN MALE MICE.

Age in days.	Number of mice.	Weight of mice in grams. (max) mean (min)	Number of glands.	Number of end-buds. (max) mean (min)	Gland area in mm ² . (max) mean (min)	Total duct length in mm. (max) mean (min)
1	14	(1.65) 1.51 (1.37)	28	(58) 46 (36)	(0.320) 0.283 (0.189)	(9.90) 8.82 (5.85)
4	12	(1.90) 1.78 (1.68)	24	(60) 48 (34)	(0.443) 0.343 (0.280)	(14.10) 10.53 (8.05)
6	12	(3.60) 3.13 (2.85)	24	(60) 47 (40)	(0.450) 0.411 (0.375)	(14.25) 12.55 (11.1)
8	18	(3.65) 3.40 (3.05)	36	(51) 44 (40)	(0.434) 0.377 (0.304)	(12.15) 10.78 (9.30)
10	15	(4.00) 3.78 (3.45)	30	(43) 40 (36)	(0.521) 0.507 (0.468)	(12.6) 11.70 (10.2)
14	15	(6.35) 5.91 (5.25)	30	(44) 40 (36)	(0.535) 0.506 (0.413)	(13.80) 11.65 (9.60)
21	18	(7.80) 7.17 (6.90)	36	(44) 41 (36)	(0.820) 0.768 (0.705)	(14.52) 13.27 (12.00)

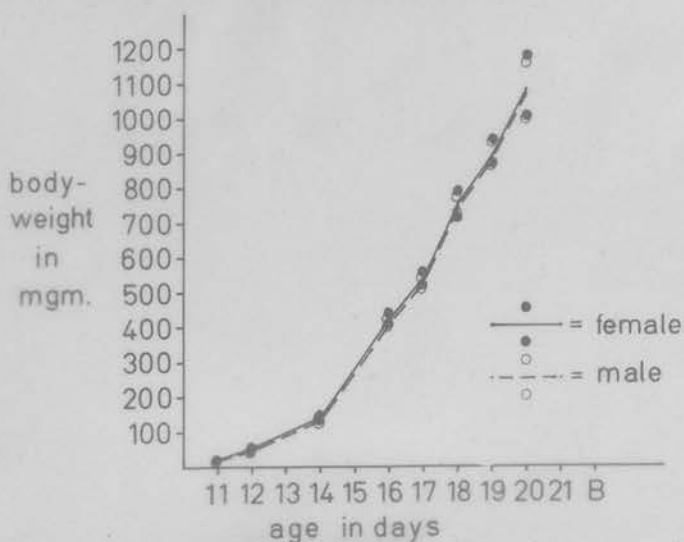
TABLE 4

COMPOSITE TABLE OF MEASUREMENTS RELATING TO THE POST-NATAL DEVELOPMENT OF THE MAMMARY GLAND IN FEMALE MICE.

Age in days.	Number of mice.	Weight of mice in grams. (max) mean (min)	Number of glands.	Number of end-buds. (max) mean (min)	Gland area in mm ² (max) mean (min)	Total duct length in mm. (max) mean (min)
1	7	(1.55) 1.43 (1.35)	14	(25) 17 (8)	(0.113) 0.063 (0.038)	(3.50) 1.82 (1.14)
4	8	(1.85) 1.71 (1.60)	16	(19) 15 (8)	(0.076) 0.059 (0.031)	(2.10) 1.63 (0.76)
6	15	(2.80) 2.63 (2.45)	30	(20) 16 (10)	(0.141) 0.106 (0.070)	(2.99) 2.48 (1.87)
8	10	(3.50) 3.21 (2.65)	20	(22) 16 (7)	(0.175) 0.108 (0.043)	(3.60) 2.64 (1.03)
10	12	(4.40) 4.07 (3.80)	24	(21) 13 (9)	(0.121) 0.070 (0.045)	(3.81) 1.96 (1.22)
14	19	(7.15) 6.50 (5.85)	38	(19) 12 (7)	(0.142) 0.090 (0.053)	(4.30) 2.76 (1.30)
21	33	(7.45) 6.88 (6.20)	66	(14) 10 (7)	(0.164) 0.094 (0.069)	(3.71) 2.50 (1.91)

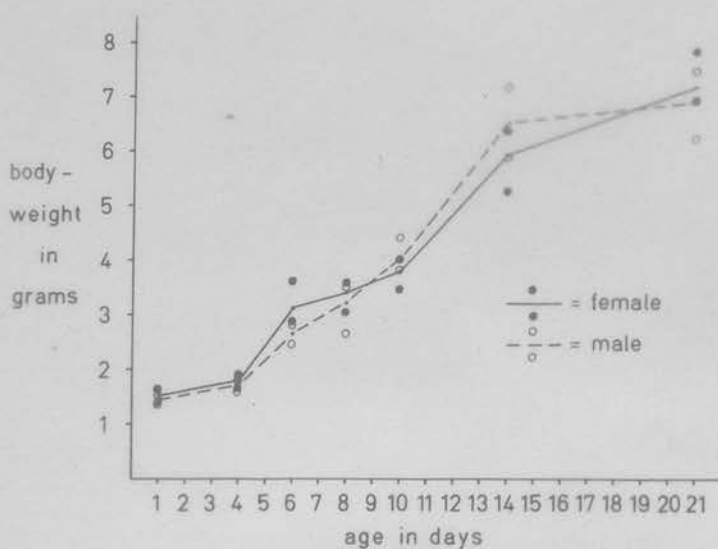
TABLE 5

COMPOSITE TABLE OF MEASUREMENTS RELATING TO THE POST-NATAL DEVELOPMENT OF THE MAMMARY GLAND IN MALE MICE.



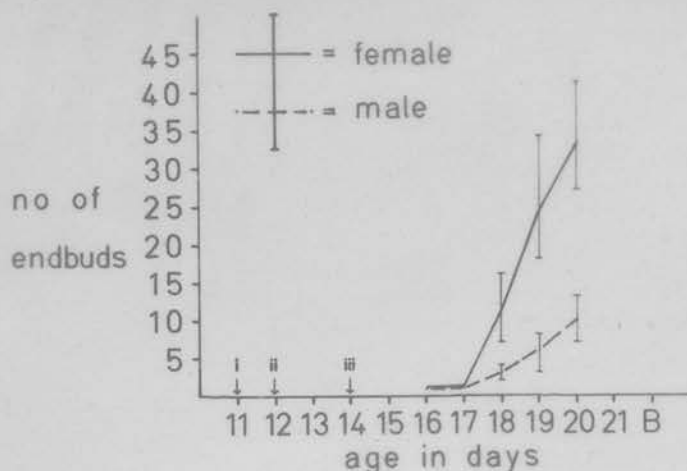
GRAPH. 1

Graph relating body weight and age in male and female mice during prenatal development. The mean and range in body weight are shown for each age studied.



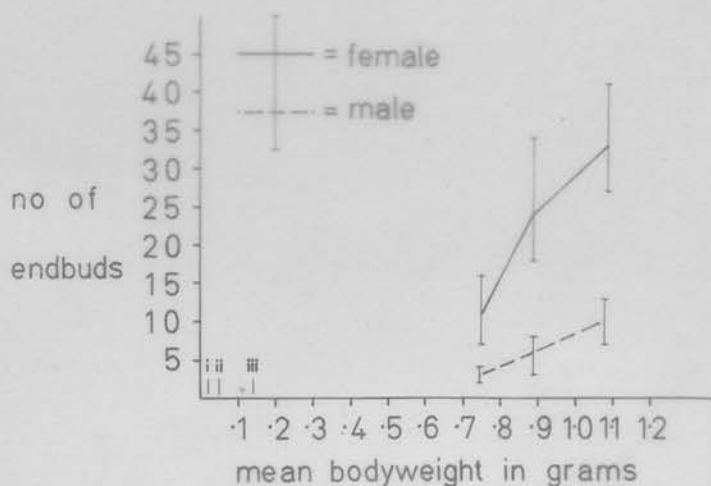
GRAPH. 2

Graph relating body weight and age in male and female mice during postnatal development. The mean and range in body weight are shown for each age studied.



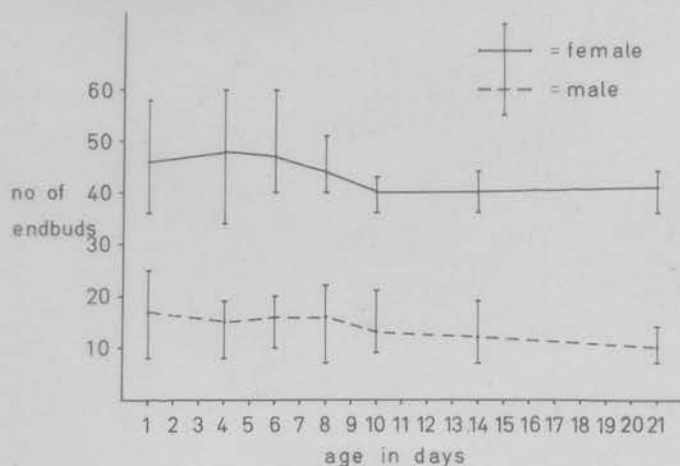
GRAPH. 3

Graph relating number of end-buds and age in male and female mice during prenatal development. The mean and range in number of end-buds are shown for each age studied.



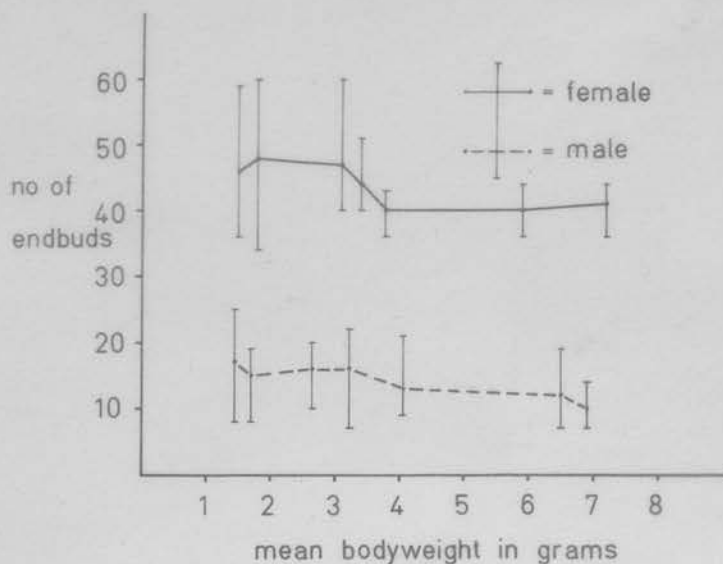
GRAPH. 4

Graph relating number of end-buds and mean bodyweight in male and female mice during prenatal development. The mean and range in number of end-buds are shown for each weight studied.



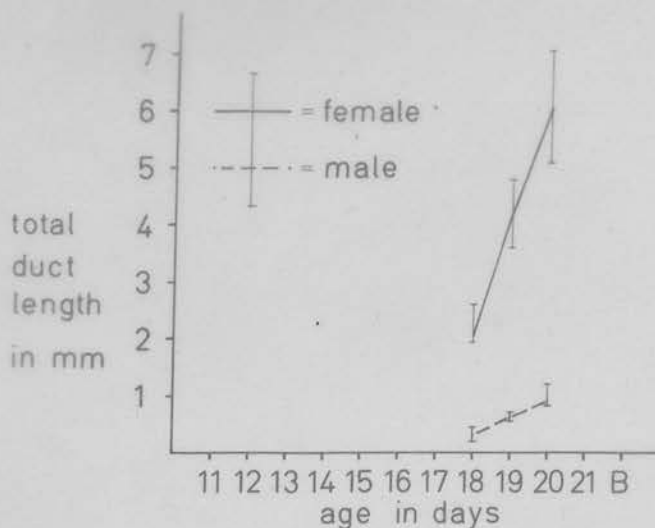
GRAPH. 5

Graph relating number of end-buds and age in male and female mice during postnatal development. The mean and range in number of end-buds are shown for each age studied.



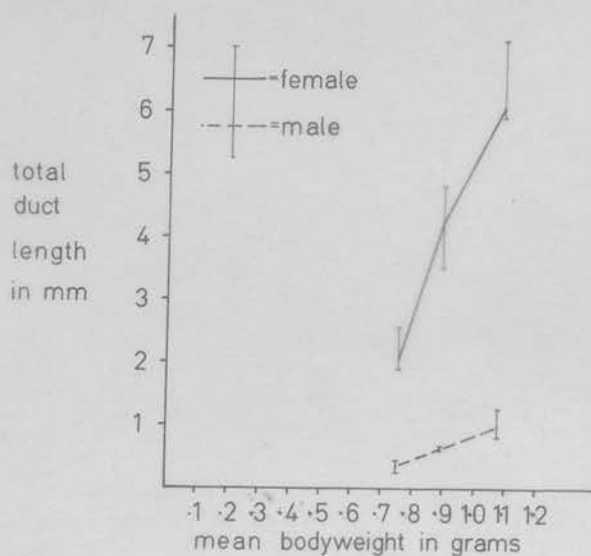
GRAPH. 6

Graph relating number of end-buds and mean bodyweight in male and female mice during postnatal development. The mean and range in number of end-buds are shown for each weight studied.



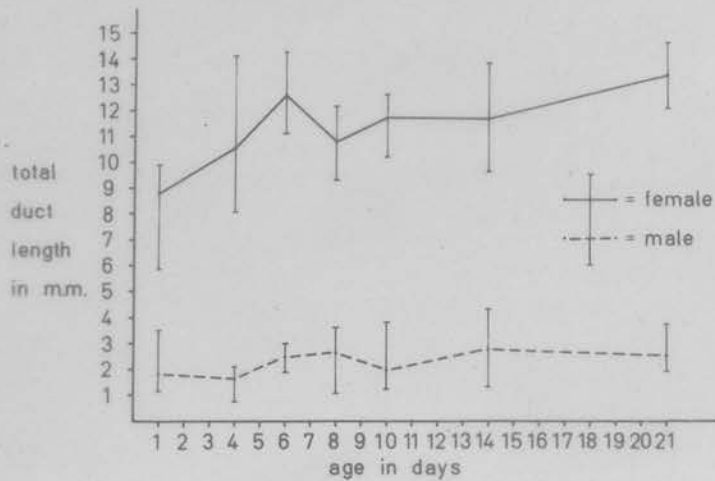
GRAPH. 7

Graph relating the total duct length and age in male and female mice during prenatal development. The mean and range in total duct length are shown for each age studied.



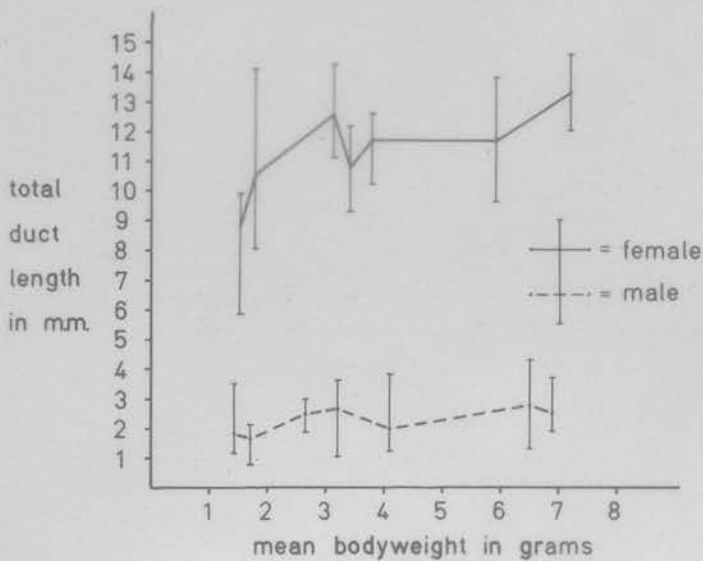
GRAPH. 8

Graph relating the total duct length and mean bodyweight in male and female mice during prenatal development. The mean and range in total duct length are shown for each weight studied.



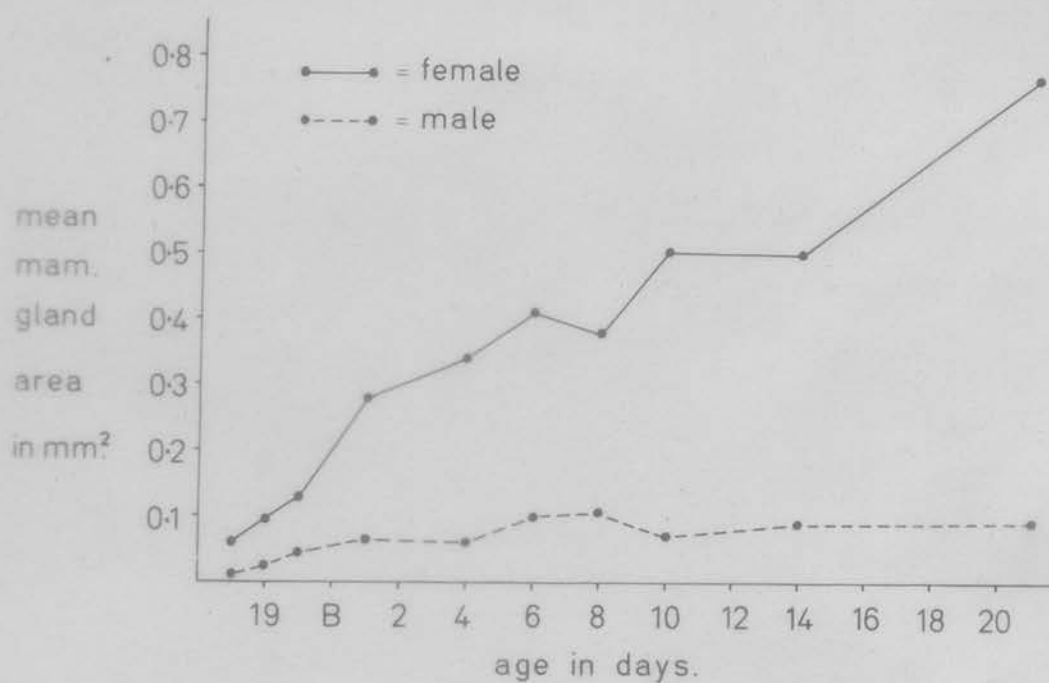
GRAPH. 9

Graph relating the total duct length and age in male and female mice during postnatal development. The mean and range in total duct length are shown for each age studied.



GRAPH. 10

Graph relating the total duct length and mean body weight in male and female mice during postnatal development. The mean and range in total duct length are shown for each body weight studied.



GRAPH. 11

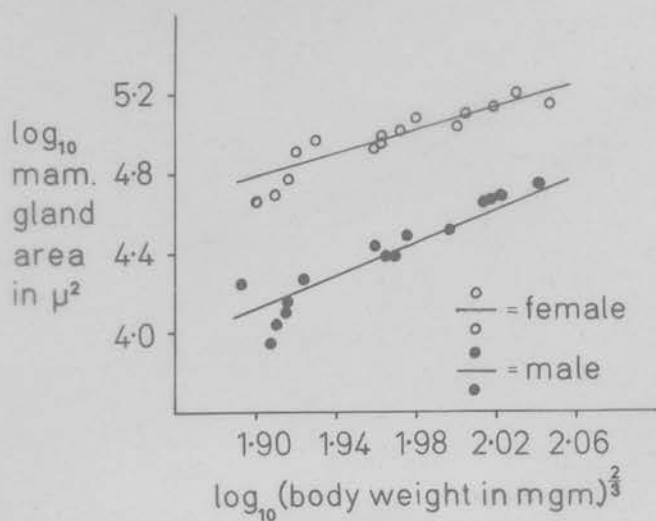
Graph relating the mean mammary gland area and age in male and female mice during prenatal and postnatal development.

FEMALE		MALE	
<u>PRENATAL</u>			
WEIGHT IN MGM.	AREA IN U ²	WEIGHT IN MGM.	AREA IN U ²
712.5	45000	700.0	18000
735.0	50000	731.0	9000
752.0	59000	737.0	11000
760.0	80000	750.0	13000
785.0	93000	758.0	14000
875.0	85000	768.0	19000
880.0	90000	872.0	28000
880.0	95000	885.0	25000
910.0	105000	900.0	24000
935.0	120000	925.0	31000
1005.0	105000	994.0	32000
1035.0	110000	1050.0	45000
1074.0	124000	1065.0	47000
1135.0	145000	1086.0	48000
1175.0	143000	1156.0	55000
<u>POSTNATAL</u>			
WEIGHT IN G.	AREA IN U ²	WEIGHT IN G.	AREA IN U ²
1.37	189000	1.35	38000
1.51	283000	1.43	63000
1.65	320000	1.55	113000
1.68	280000	1.60	31000
1.78	343000	1.71	59000
1.90	443000	1.85	76000
2.85	375000	2.45	70000
3.05	304000	2.63	106000
3.13	411000	2.65	43000
3.40	377000	2.80	141000
3.45	468000	3.21	108000
3.60	450000	3.50	175000
3.65	434000	3.80	45000
3.78	507000	4.07	70000
4.00	521000	4.40	121000
5.25	413000	5.85	53000
5.91	506000	6.20	69000
6.35	535000	6.50	90000
6.90	705000	6.88	94000
7.17	768000	7.15	142000
7.80	820000	7.45	164000

TABLE 6

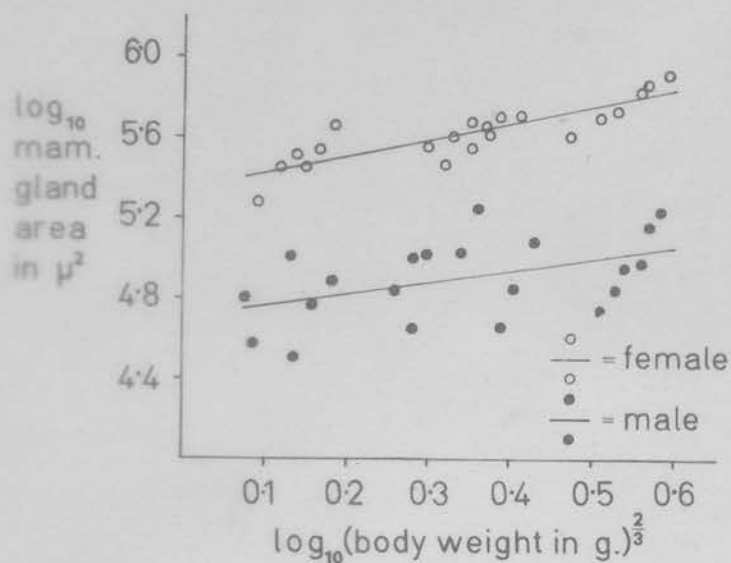
COMPOSITE TABLE OF MEASUREMENTS RELATING TO THE RELATIVE GROWTH ANALYSIS OF THE MALE AND FEMALE MAMMARY GLANDS DURING PRENATAL AND POSTNATAL DEVELOPMENT.

The weight measurements represent the body weight of each animal studied. The area measurements represent the mean outline gland area of paired glands in each animal studied.



GRAPH. 12

Graph relating mammary gland area and $(\text{body weight})^{2/3}$ in the male and female mouse during prenatal development. Logarithmic plotting. All points used in the calculation of the regression lines.



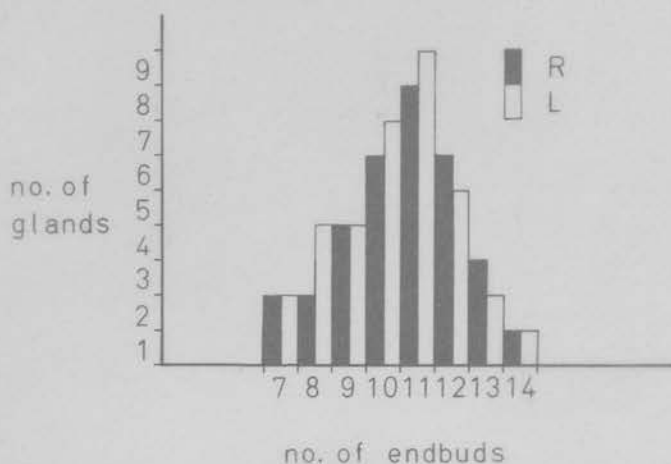
GRAPH. 13

Graph relating mammary gland area and $(\text{body weight})^{2/3}$ in male and female mice during postnatal development. Logarithmic plotting. All points used in the calculation of the regression lines.

MALES				FEMALES			
NO.	R.	L.	L-R.	NO.	R.	L.	L-R.
1	10	11	+1	1	37	41	+4
2	12	10	-2	2	40	42	+2
3	9	10	+1	3	39	41	+2
4	13	11	-2	4	43	41	-2
5	11	9	-2	5	36	43	+7
6	12	12		6	46	42	-4
7	9	11	+2	7	41	41	
8	10	10		8	39	38	-1
9	13	12	-1	9	42	39	-3
10	11	14	+3	10	42	42	+1
11	11	11		11	41	39	-2
12	10	11	+1	12	40	40	
13	13	12	-1	13	39	41	+2
14	14	11	-3	14	43	42	-1
15	7	9	+2	15	42	40	-2
16	11	8	-3	16	41	38	-3
17	12	10	-2	17	38	40	+2
18	9	12	+3	18	40	39	-1
19	11	11		19	39	42	+3
20	12	10	-2	20	42	42	
21	8	10	+2	21	44	40	-4
22	9	8	-1	22	40	43	+3
23	11	10	-1	23	37	37	
24	10	12	+2	24	41	39	-2
25	11	11		25	43	42	-1
26	8	9	+1	26	45	40	-5
27	12	9	-3	27	44	44	
28	11	11		28	43	44	+1
29	10	13	+3	29	38	37	-1
30	10	8	-2	30	42	44	+2
31	12	13	+1	31	41	45	+4
32	7	7		32	41	36	-5
33	8	7	-1	33	41	41	
TOTAL NO.	346	343	-3	1350	1346	-4	
MEAN NO.	10.48	10.40	-0.08	40.90	40.78	-0.12	

TABLE 7

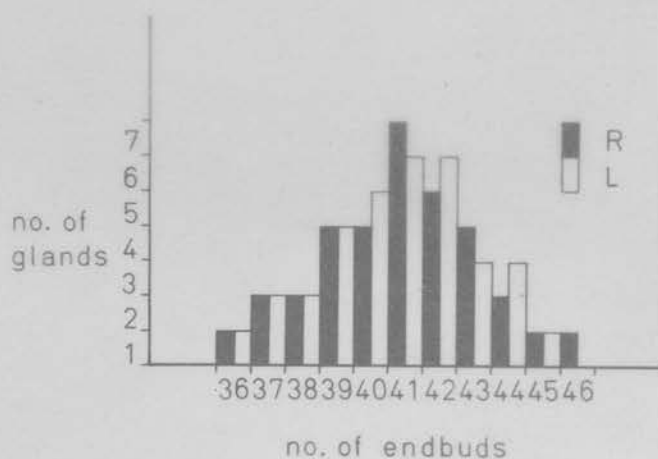
COMPARISON OF THE NUMBER OF END-BUDS BETWEEN THE RIGHT AND LEFT MAMMARY GLANDS OF BOTH MALE AND FEMALE MICE AGED 21 DAYS.



HISTOGRAM 1

Histogram showing the number of end-buds in the Right and Left mammary glands of male mice aged 21 days.

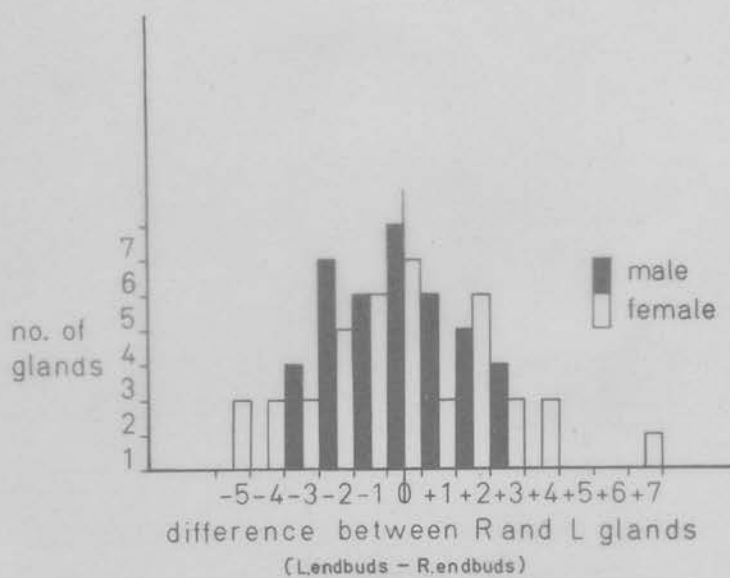
(Total number of glands studied = 66)



HISTOGRAM 2

Histogram showing the number of end-buds in the Right and Left mammary glands of female mice aged 21 days.

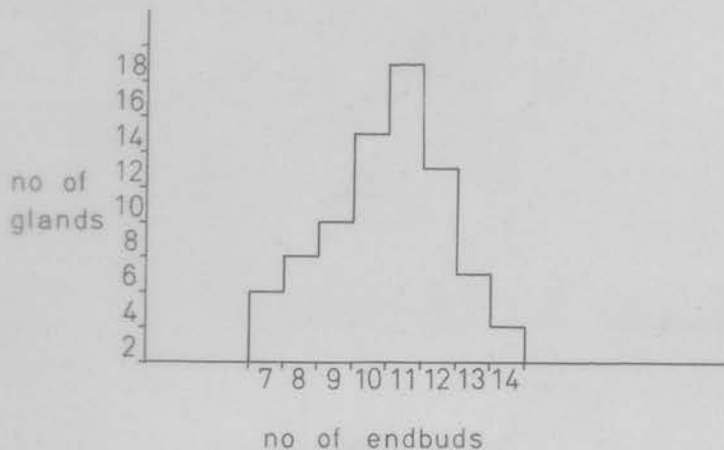
(Total number of glands studied = 66)



HISTOGRAM 3

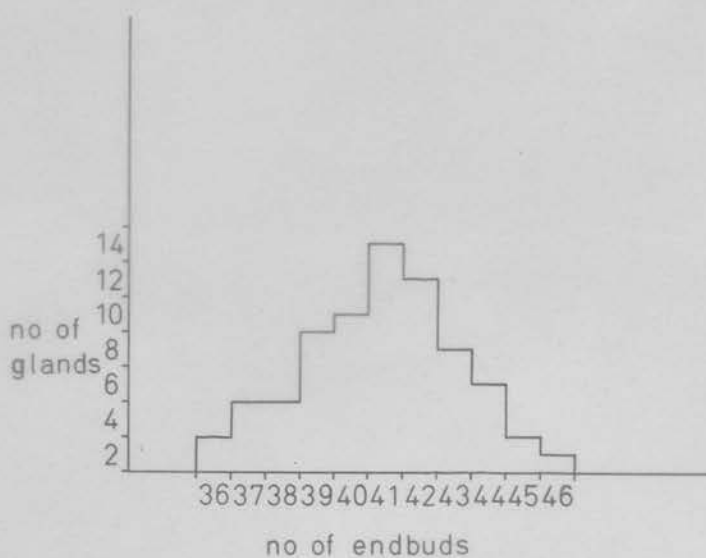
Histogram showing the difference in the number of end-buds in paired mammary glands of male and female mice aged 21 days.

(Total number of glands studied = 66 pairs)



HISTOGRAM 4

Histogram showing the number of end-buds in the mammary glands of male mice aged 21 days.
(Total number of glands studied = 66)



HISTOGRAM 5

Histogram showing the number of end-buds in the mammary glands of female mice aged 21 days,
(Total number of glands studied = 66)

2. Experimental development study.

I. Serial sections and whole mounts.

(i) Cultures of third thoracic 16 day embryonic gland rudiments in Trowells T8 medium only.

(a) Male and female glands (Grown separately and together) (figs. 64a, 64b and 65).

In all cultures the organotypic structure of the mammary bud is maintained and good cellular survival is confirmed by the absence of evidence of cell death such as pyknosis, karyorrhexis and karyolysis. None of the gland rudiments has undergone any significant developmental differentiation.

(ii) Cultures of third thoracic 16 day embryonic gland rudiments in Trowells T8 medium + Male embryo extract + Maternal serum.

(a) Male glands (Grown separately and with female glands) (figs. 66 and 70). The organotypic structure is maintained. The gland rudiments have developed along the normal male mammogenetic pattern. Complete separation from the skin has occurred in all glands which lie parallel to the skin surface displaying a few solid end-buds. Canalization has started in the primary sprout in some cases. Endothelial lined spaces can be seen in the region of the growing sprout. These are of uncertain nature but may be vascular in origin.

(b) Female glands (Grown separately and with male glands) (figs. 67 and 72). The organotypic structure is maintained. The gland rudiments have almost completely followed the male histogenetic pattern. Only in one gland (fig. 72) is separation from the epidermis incomplete. All other rudiments lie/

lie deep to the epidermis with no attachment to it but with the characteristic mesenchymal condensation between the proximal end of the gland and the epidermis. There is no evidence of nipple formation. Most glands display typical short epithelial end-buds characteristic of the male gland. Mitotic figures can be seen in these end-buds. In some cases, canalization has started in the primary sprouts.

(iii) Cultures of third thoracic 16 day embryonic gland rudiments in Trowells T8 medium + Female embryo extract + Maternal serum.

(a) Male glands (Grown separately and with female glands) (figs. 68 and 71). Organotypic structure is maintained. The gland rudiments have followed a female histogenetic pattern. The primary sprout is still in contact with the epidermis by means of a constricted neck, and runs parallel to the skin surface laterally. In most cases, the primary sprout divides into two secondary sprouts which in turn give rise to tertiary sprouts ending quickly in short solid epithelial end-buds. In some glands, however (fig. 71) this typical female branching does not occur and branching continues in an atypical fashion. A well formed epithelial mammary cap overlies the primary sprout but the epithelial downgrowth of the deeper layer of the epidermis characteristic of the female epithelial hood is not present. Some areas of canalization can be seen along the primary and secondary sprouts. Capillaries and other wider endothelial lined spaces can be seen in the immediate surroundings of the sprouts.

(b) Female glands (Grown separately and with male glands) (figs/

(figs. 69 and 73). The glands have maintained their organotypic structure and have developed along the normal female histogenetic pattern. The primary sprout is attached to the epidermis by means of a constricted neck, and runs laterally almost parallel to the skin surface. In all cases, the primary sprout divides into two secondary sprouts with tertiary branches which end in solid epithelial end-buds. All glands have a well formed epithelial mammary cap but the epithelial downgrowth of the deep layer of the epidermis is only present in a few glands and in these only at an early stage. Canalization has started in some areas of the primary and secondary sprout system. Wide endothelial lined spaces and capillaries can again be seen around the sprouts.

II. Quantitative analysis of experimental development.

No analysis is given of growth in Trowells T8 medium only.

Measurement relating to the experimental development in the glands grown in Trowells T8 medium + Male embryo extract + Maternal serum and in Trowells T8 medium + Female embryo extract + Maternal serum are given in Table 8. The arithmetic mean of all measurements are compared to the arithmetic mean of the same measurements in the normal control male and female embryonic glands aged 20 days in Histograms 6 to 11.

The following observations are made:

(i) Gland rudiments grown in Trowells T8 medium + Male embryo extract + Maternal serum.

(a) Number of end-buds (Histogram 6)

The female and male gland rudiments grown separately and the male gland rudiment grown with a female gland rudiment achieved/

achieved 30% of the normal number of end-buds in in vivo development. The female gland rudiments grown in the presence of male rudiments have achieved 40% of the normal number of end-buds.

(b) Duct length (Histogram 8)

Both groups of male explants have achieved approximately 50% the duct length in the control male glands whereas the female explants achieved approximately 60% of the normal male control.

(c) Outline area (Histogram 10)

Both groups of male explants have an area of approximately 30% of the area of the normal control. The female gland rudiments grown separately have an area of 40% of the normal whereas the female gland rudiments grown in the presence of male explants have an area of more than 50% of the area found in the normal control male glands.

(11) Gland rudiments grown in Trowells T8 medium + Female embryo extract + Maternal serum.

(a) Number of end-buds (Histogram 7)

Both groups of male gland rudiments have 20% of the number of end-buds found in the control female whereas the female explants of both groups have 25% of the normal number of end-buds at 20 days of embryonic life.

(b) Duct length (Histogram 9)

Both groups of male gland rudiments have achieved less than 20% of duct length of the normal female gland, whereas the female rudiments have a duct length of approximately 25% of the normal female control.

(c)/

(c) Outline area (Histogram 11)

The two groups of male explants have areas of approximately 30% of the normal female gland and the two groups of female explants have areas of approximately 40% of the normal control female gland.

Throughout this analysis the female gland explants have developed more than the male explants by approximately 10% of the normal controls in each case.

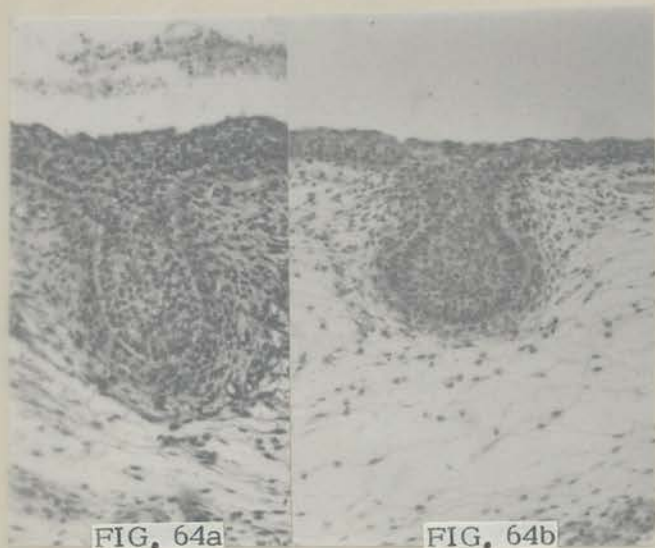


FIG. 64a

FIG. 64b

Transverse sections through mammary buds from male and female embryos aged 16 days cultured in Trowells medium only for 5 days. Male bud on right and female bud on left.

Haematoxylin and eosin, X 120

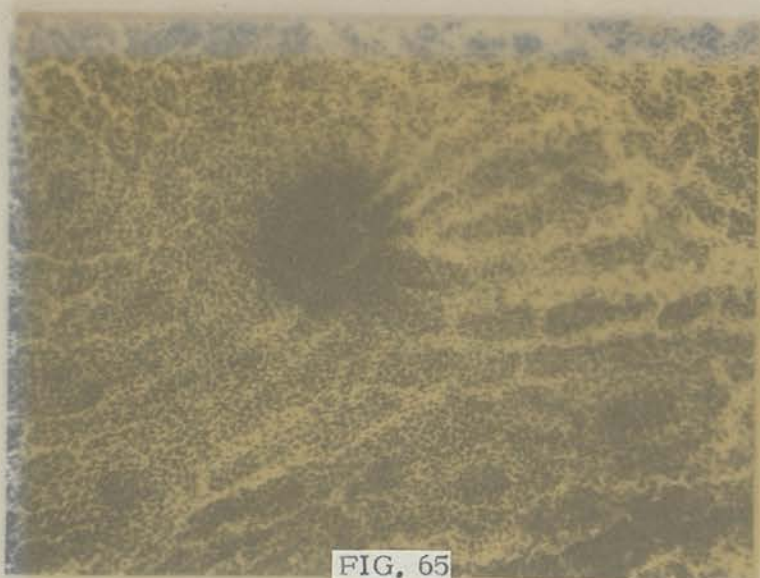


FIG. 65

Whole mount of mammary bud from female embryo aged 16 days cultured in Trowells medium only for 5 days.

Haematoxylin, X 120

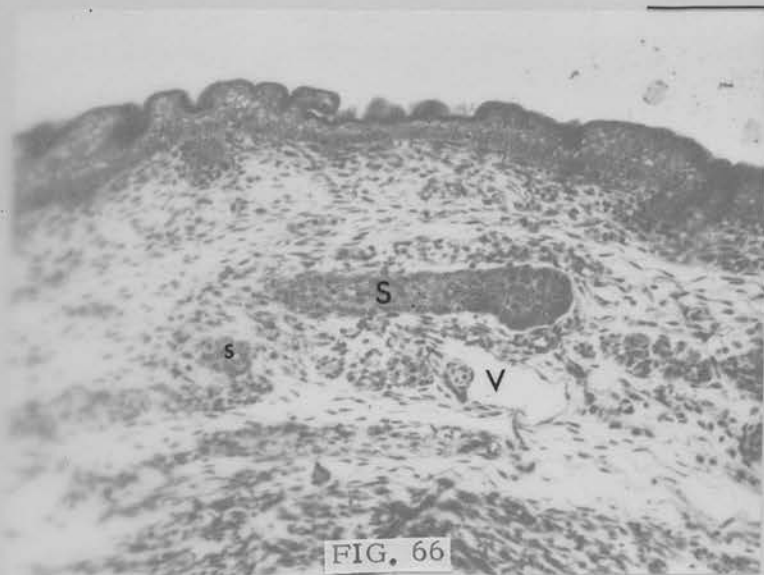


FIG. 66

Longitudinal section through mammary sprout from male embryo aged 16 days cultured in Trowells medium with male embryo extract and maternal serum for 5 days.

Haematoxylin and eosin. X 120

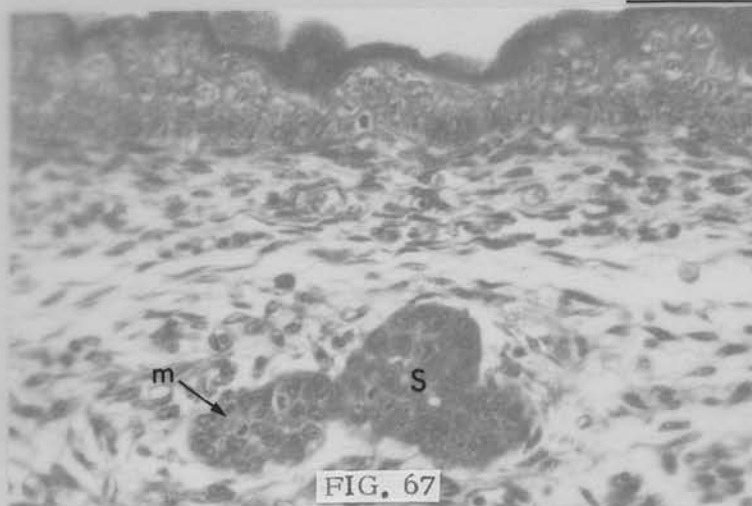


FIG. 67

Transverse section through mammary sprout from female embryo aged 16 days cultured in Trowells medium with male embryo extract and maternal serum for 5 days.

Haematoxylin and eosin. X 330

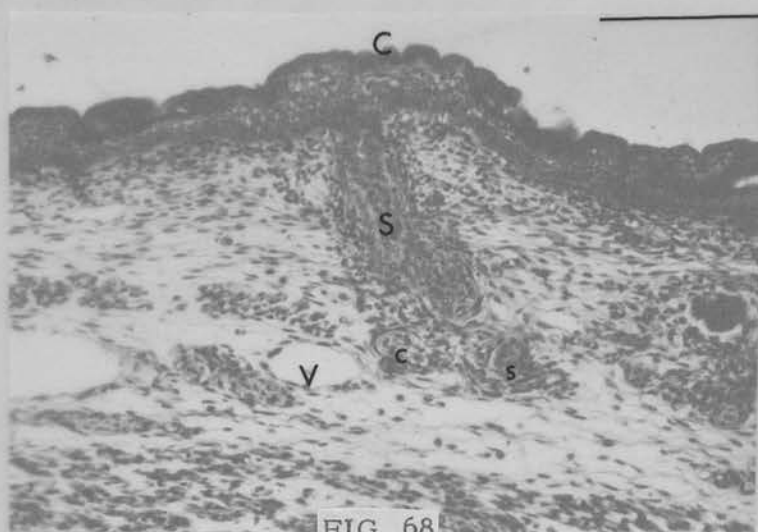


FIG. 68

Transverse section through mammary sprout from male embryo aged 16 days cultured in Trowells medium with female embryo extract and maternal serum for 5 days.

Haematoxylin and eosin,

X 120

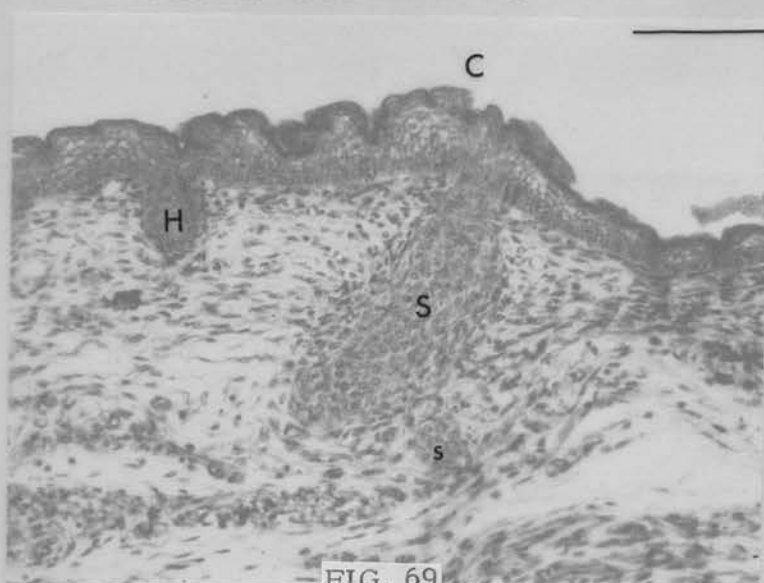


FIG. 69

Transverse section through mammary sprout from female embryo aged 16 days cultured in Trowells medium with female embryo extract and maternal serum for 5 days.

Haematoxylin and eosin,

X 120

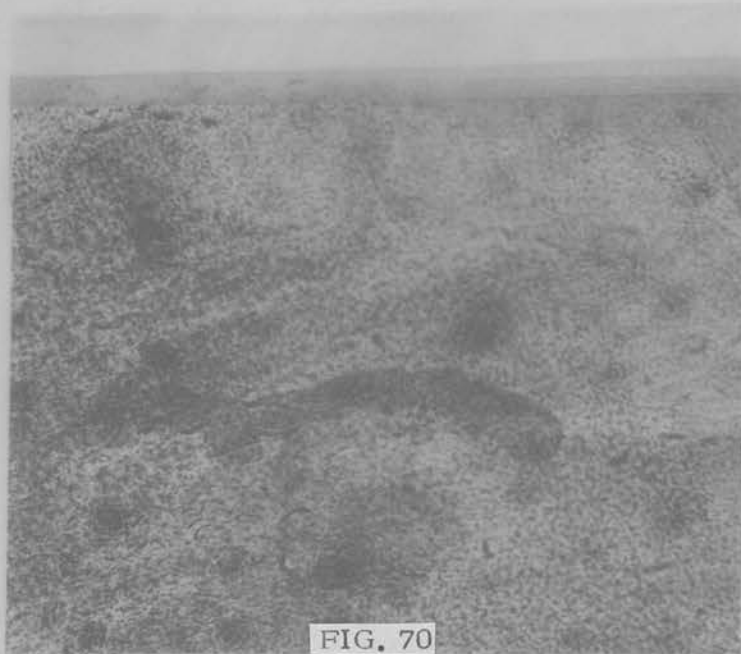


FIG. 70

Whole mount of mammary bud from male embryo aged 16 days cultured in Trowells medium with male embryo extract and maternal serum for 5 days.

Haematoxylin, X 120

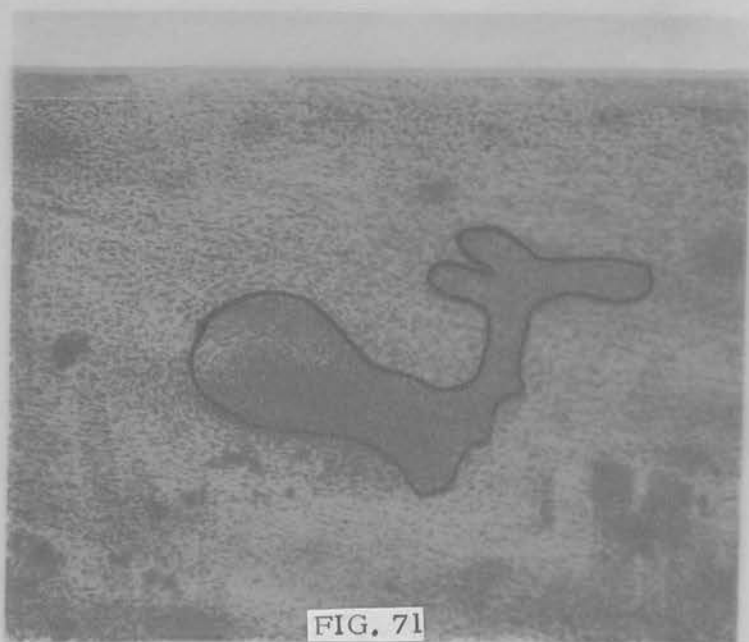


FIG. 71

Whole mount of mammary bud from male embryo aged 16 days cultured in Trowells medium with female embryo extract and maternal serum for 5 days.

Haematoxylin, X 120

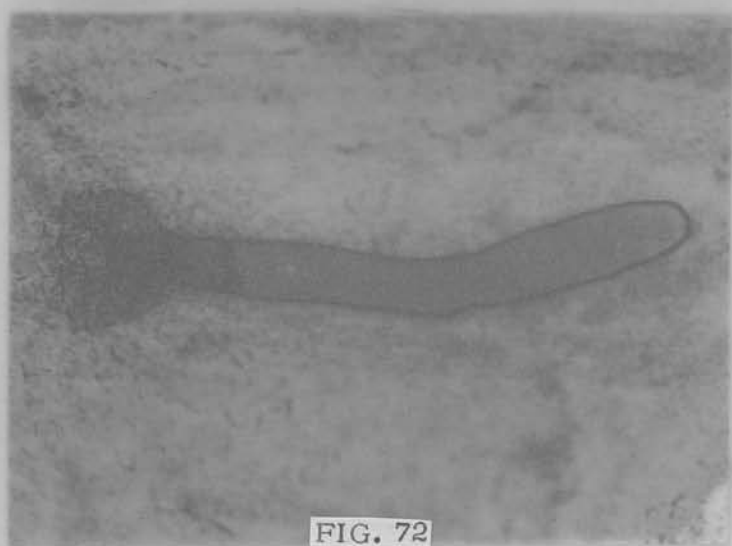


FIG. 72

Whole mount of mammary bud from female embryo aged 16 days cultured in Trowells medium with male embryo extract and maternal serum for 5 days.

Haematoxylin, X 120

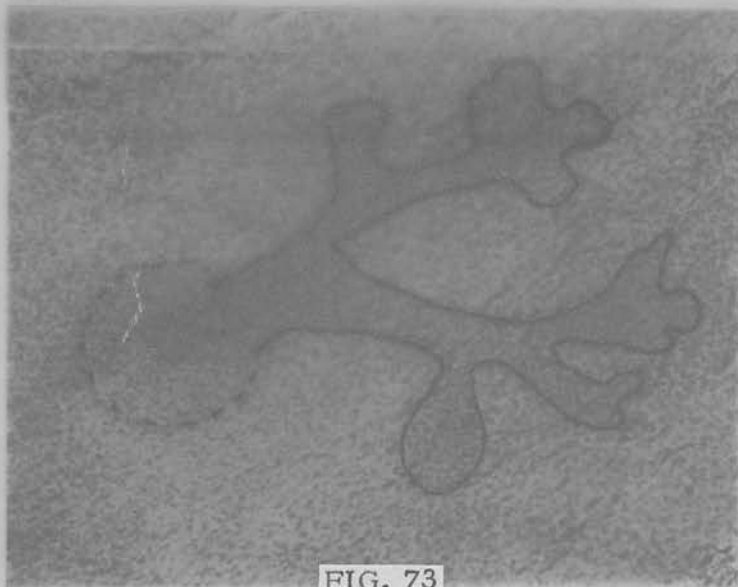


FIG. 73

Whole mount of mammary bud from female embryo aged 16 days cultured in Trowells medium with female embryo extract and maternal serum for 5 days.

Haematoxylin, X 120

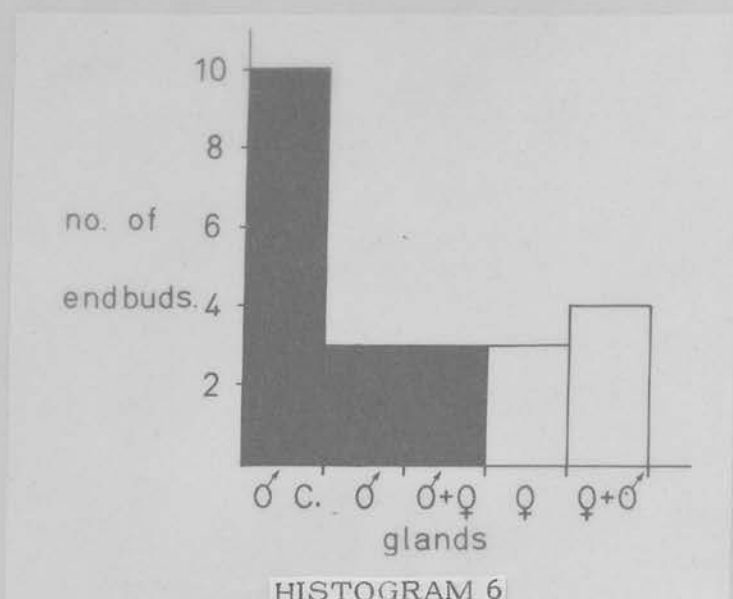
	NUMBER OF GLANDS.	NUMBER OF END-BUDS. (max) mean (min)	AREA IN SQ.MM. (max) mean (min)	DUCT-LENGTH IN MM. (max) mean (min)
I.				
MALE ALONE	7	(6) 3 (2)	(0.020) 0.015 (0.012)	(0.650) 0.525 (0.470)
MALE + FEMALE	4	(5) 3 (2)	(0.020) 0.013 (0.009)	(0.630) 0.510 (0.435)
FEMALE ALONE	6	(5) 3 (1)	(0.034) 0.020 (0.010)	(0.700) 0.600 (0.495)
FEMALE + MALE	4	(5) 4 (2)	(0.030) 0.026 (0.015)	(0.695) 0.605 (0.520)
II.				
MALE ALONE	8	(11) 6 (4)	(0.049) 0.036 (0.025)	(0.820) 0.756 (0.710)
MALE + FEMALE	6	(9) 6 (4)	(0.045) 0.039 (0.021)	(0.810) 0.765 (0.705)
FEMALE ALONE	8	(12) 8 (5)	(0.065) 0.050 (0.036)	(1.900) 1.355 (1.010)
FEMALE + MALE	6	(11) 8 (5)	(0.060) 0.052 (0.039)	(1.850) 1.400 (1.075)

TABLE 8

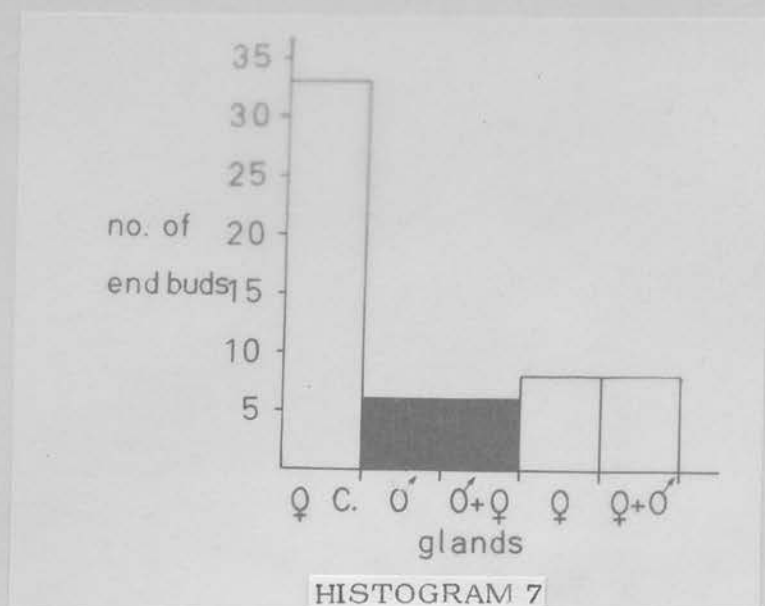
COMPOSITE TABLE OF MEASUREMENTS RELATING TO THE IN VITRO DEVELOPMENT OF THE MALE AND FEMALE MAMMARY GLANDS.

I. Cultured in Trowells T8 medium + Male embryo extract + Maternal serum.

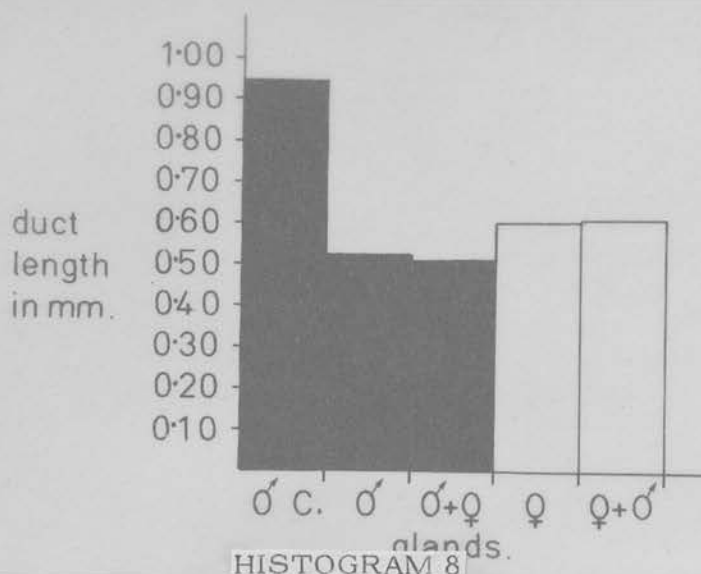
II. Cultured in Trowells T8 medium + Female embryo extract + Maternal serum.



Histogram showing the mean number of endbuds in the control embryonic male glands aged 20 days and the male and female glands cultured in Trowell's T8 medium + male embryo extract + maternal serum.

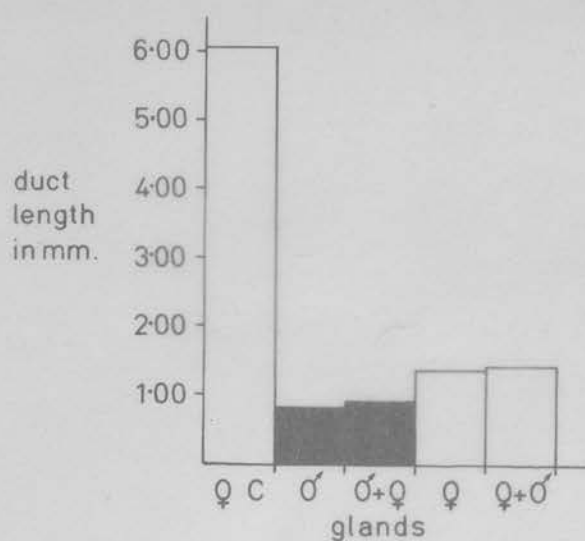


Histogram showing the mean number of endbuds in the control embryonic female glands aged 20 days and the male and female glands cultured in Trowell's T8 medium + female embryo extract + maternal serum.



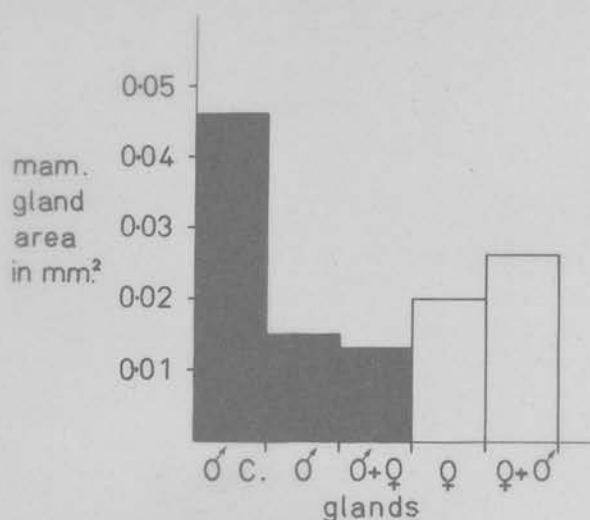
HISTOGRAM 8

Histogram showing the mean duct length in the control embryonic male glands aged 20 days and the male and female glands cultured in Trowells T8 medium + male embryo extract + maternal serum.



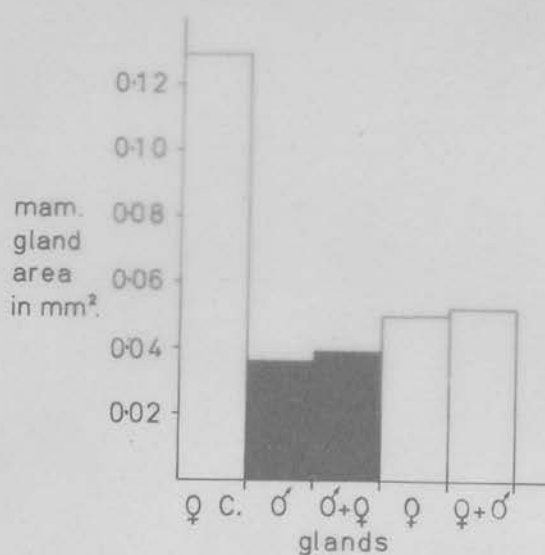
HISTOGRAM 9

Histogram showing the mean duct length in the control embryonic female glands aged 20 days and the male and female glands cultured in Trowells T8 medium + female embryo extract + maternal serum.



HISTOGRAM 10

Histogram showing the mean mammary gland area in the control embryonic male glands aged 20 days and the male and female glands cultured in Trowells T8 medium + male embryo extract + maternal serum.



HISTOGRAM 11

Histogram showing the mean mammary gland area in the control embryonic female glands aged 20 days and the male and female glands cultured in Trowells T8 medium + female embryo extract + maternal serum.

DISCUSSION.

Only a few systematic studies have been carried out on the embryogenesis of the mammary gland in the mouse and all of them describe only the histological changes during embryonic development. The most useful studies are those of Gibson (1930) who only studied the female gland, Turner and Gomez (1933), and Raynaud (1947d) who report on sexual differentiation, and Balinsky (1950) who makes no mention of sex at all in his study. Gibson (1930) and Turner and Gomez (1933) also consider the postnatal development with emphasis on puberty, oestrus, pregnancy, lactation and involution, and their work is followed by the studies of Gardner and Strong (1935) and Richardson and Cloudman (1947) on adult mice. In these studies whole mount techniques are also employed to study overall changes.

Other studies have been carried out on the rat (Henneberg 1900; Myers 1917; Moon et al 1959), the rabbit (Ancel and Bouin 1911), the monkey (Aberle 1934b; Speert 1948) and Turner carried out a series of investigations on the mammary gland development in various animals including the guinea pig, goat and calf and gives a full account of the main developmental features in several species with variations in a valuable monograph (Turner 1939). The human mammary gland development has been studied extensively particularly by the German anatomists of the late 19th century (Langer 1871; Huss 1873; Kölliker 1879; Rein 1881-2; Schultze 1892; Bonnet 1892; Strahl 1898 and Hirschland 1899). A detailed review and account of the development of the human mamma is given by Dawson (1934).

1. Normal development study.

I. Serial sections.

The development of the mammary gland in the C57BL^t/H mouse strain shows agreement in most aspects with the above mentioned studies on other mouse strains but some differences can be found. Variations in the time of the appearance of the mammary rudiment are seen between different strains. Turner and Gomez (1933) find the earliest indication of the albino mouse mammary gland rudiment on the tenth day of embryonic life, whereas Gibson (1930) in her study of two albino mouse strains states that it appears shortly after the tenth day. Raynaud (1947d) describes the appearance of the mammary bud in the XXX strain of mice at twelve days ten hours and Balinsky (1950) accepts day eleven as the time of onset of mammary development in the heterogenous mice of his investigation. These variations are probably due to a combination of strain differences and the techniques used to determine the onset of pregnancy. The localized origin of the mammary rudiment in the strain of mice used in this study agrees with the descriptions given by Raynaud (1947d) of the inguinal gland rudiments in the XXX strain of mice, and by Balinsky (1950) of the appearance of the thoracic mammary glands in the heterogenous strain of mice which he studied. The present findings do not confirm the presence of a mammary streak and line such as that described in the albino mouse (Gibson 1930; Turner and Gomez 1933) and albino rat (Henneberg 1900; Myers 1917). Histologically the early developmental changes in the localized regions of the epidermis correspond to the descriptions of similar changes occurring in the mammary streak and line of the/

the albino mouse and rat, and also to the changes in the mammary band of the human developing breast (Schultze 1892; Kallius 1897; Strahl 1898 and Hirschland 1899).

The mammary hillock which is found in all species so far described was originally said to be hill-like ("hügelförmig") by Langer (1871), Huss (1873), Gegenbauer (1876), Kölliker (1879) and Rein (1881), while Bonnet (1892) called it the milk hillock (milch-hügel), Schultze (1892) described it as a primitive teat and Kallius (1897) called it a mammary crest.

The identical differentiating processes leading to the formation of the mammary sprout and the persistence of the mammary sprout for some time in both sexes, correspond closely in all the mouse strains described and support the quantitative study of Balinsky (1950) on the mitotic index of the gland rudiment, which shows that this early development consists of a period of relatively slow growth accompanied by an absence of further progressive differentiation. That this phase of slow development is followed by a phase of more rapid growth and cellular proliferation as found by Balinsky (1950), is shown clearly in this study by the presence of numerous mitoses during this stage and later by the rapid changes observed in the whole mount preparations.

Sexual differentiation in the present study is indicated by the male sprout separating from the epidermis at 17 days of embryonic life and remaining separate more or less rudimentary in its further development. This confirms the pattern described in the albino and XXX mouse strains by Turner and Gomez (1933) and Raynaud (1947d), but in these two strains sexual differentiation/

differentiation starts at 16 days in the albino mouse and at 15 days 15 hours in the XXX strain.

The formation of the epithelial hood apparently starts on the 18th day in the female albino embryo (Turner and Gomez 1933). Gibson (1930) and Raynaud (1947d) do not describe the nipple formation in detail in their studies. The further development of the nipple in the female albino (Turner and Gomez 1933), is identical with the strain in this study.

Canalization starts at approximately the same period in all strains described.

The postnatal development in the females of all the strains described corresponds closely, with no appreciable difference in overall structure or histology. Strain differences in the relative growth of the mammary glands and in the sensitivity of the mammary glands to various exogenous hormones in female mice have however, been reported by several authors in recent years (Ranadive 1945; Richardson 1955; Nagai et al 1957; Nandi 1961; Yoshida 1963).

The postnatal development of the male mammary gland in the C57BL^a/H strain corresponds with that of the albino mouse (Turner and Gomez 1933), however, the studies of Richardson and Cloudman (1947) and Richardson (1951; 1953) on the mammary glands of male mice aged 9 weeks in 11 pure strains and F₁ hybrids of reciprocal crosses between 4 of these strains, show that strain differences exist in the male mammary glands as well. These consist of differences in the size and structure of the mammary glands, varying from large mammae in the pure Leaden strain and F₁ hybrids of the Leaden and 3 other strains, and glands which are/

are either small, or rudimentary, or even absent more especially in the Ak/r, C, Bagg and dba male strains.

II. The quantitative analysis of mammary gland development.

(i) Whole mount analysis.

The examination of thin sections taken from sample areas of a structure like the mammary gland can, at the very best, give only an incomplete and often misleading picture of the growing duct system. Laborious reconstructions from sections give a better three dimensional picture but have many disadvantages. The flat, uniplanar, almost two dimensional distribution of the mouse thoracic mammary gland duct systems makes this method unnecessary. The technique of making whole mount preparations of the mammary glands originally developed by Lane-Claypon and Starling (1906), is a much more useful method for studying the overall structure of the gland. This technique, which is particularly suitable in animals such as the mouse, rat, rabbit and rhesus monkey with flat mammary gland systems (Richardson 1947), was adopted by Ancel and Bouin (1911) for their studies on the rabbit mammary gland. Aberle (1934a) pointed out early that shrinkage occurs during the preparation of whole mounts. She estimated that whole mounts of the mammary glands of the rhesus monkey by the Lane-Claypon Starling technique underwent shrinkage of approximately 7%. At the same time, she noted that the mammary gland of the rhesus monkey grows in length and width rather than in thickness, and concluded that measurements of the outline gland area from the whole mount preparations would give a good index of changes in size during growth. This uniplanar distribution of the mammary gland also found in the/

the thoracic glands of the mouse, prompted authors during the last 30 years to use the whole mount technique in studies on these glands. (Gibson 1930; Turner and Gomez 1933; Hadfield and Young 1956).

Folley et al (1939) following up the work of Aberle (1934a), made area measurements for quantitative analyses on the mammary gland of the rhesus monkey. However, in view of the work of Zuckermann (1932) from which he (Zuckermann) concluded that there is no correlation between the size and functional activity of the mammary glands of the green monkey (*cercopithecus sabacus*), Folley and his colleagues (1939) stressed that data obtained from whole mount preparations had to be supplemented by macroscopical and histological examination of the gland structure. Du Bois (1941; 1944) appreciating the full value of this method in quantitative analyses of mammary gland development, but realizing its limitations, describes in detail the precautions necessary in preparing whole mounts of the mammary gland in the mouse.

With increased interest in the effects of exogenous and endogenous factors on the mammary gland development, whole mount analysis is now used extensively.

The following measurements can be obtained from a whole mount preparation of the mammary gland:

(a) The number of end-buds, duct-buds, end-clubs or alveoli.

As growth occurs by ramification of the mammary tree, due to budding of a number of off-shoots which eventually terminate in end-buds, clubs or alveoli depending on the age of the animal and whether it is pregnant or not (Richardson 1947), the recording/

recording of the number of terminations present in a gland is an accurate index of the state of its development. It is a simple and accurate measurement presenting almost no difficulty apart from being able to distinguish a developing duct-bud appearing as a prominent hemispherical swelling in the duct wall. Its accuracy diminishes when it is applied to the oestrus, pregnant or fully active lactating gland because of the degree of ramification of the gland which obscures the actual number of terminations. In the embryonic or prepubertal gland used in this study, the number of end-buds provides a good quantitative measurement for comparing either male and female glands with each other in normal development, or with similar glands exposed to experimental procedures (the normal glands acting as controls), provided that a stable strain of mice is used throughout. This measurement has been used extensively particularly in experimental studies on the response of the breast bud-formation to mammotrophic stimuli (Hadfield and Young 1956). It is the main measurement in this study for comparing mammary growth in paired glands, in male and female glands in vivo, and in male and female glands grown in culture. The relation of the number of end-buds to both age and body weight in all cases provides accurate quantitative information.

(b) Total duct length.

This is also an easy method with a high degree of accuracy (Nicoll 1965). Shrinkage is a possible source of distortion. As all whole mounts are prepared by a standardized technique, the proportional shrinkage should be the same in all glands (male or female, prenatal or postnatal), but the actual shrinkage will be more/

more obvious in the larger postnatal female glands. Relating the total duct length to both age and body weight provides useful quantitative information on the mammary gland development (normal and experimental) in this study.

(c) Ductal extension.

Hoshino (1962) in his comparative study of transplanted and control inguinal glands in mice, estimated the "ductal extension" of the glands as an index of growth potentiality. He made the following measurements:

The distance between the nipple and the ventro-medial border of the ventral inguinal lymphnode.

The distance between the nipple and the farthest distal end of the mammary duct.

The greatest extent of mammary growth i.e. the widest diameter of the gland.

This method is obviously inaccurate in many ways (Nicoll 1965). Variations in the exact position of the lymphnodes must occur and the technique of stretching the pelt on cork before fixation and staining as described by Hoshino (1962), must alter the measurements considerably.

(d) Mammary gland area measurements.

The measurement of the outline mammary gland area by some or other method has always featured in quantitative studies of the breast. Unfortunately, it has many inaccuracies which limits its usefulness. In efforts to overcome these limitations, several different techniques have been developed.

A very misleading method is employed by Folley et al (1939)/

(1939) in their estimation of the mammary gland area of the male rhesus monkey. They take the area of the male gland as the area enclosed by a line joining the extremities of the ducts. This method is also used by Blair et al (1957) in a study on the male mouse breast. A modification of this technique is adopted by Richardson and Cloudman (1947) and Richardson F(1951; 1953) who examine whole mount preparations of mouse mammary glands under a dissecting microscope and with the aid of calipers measure the length and the greatest width at right angles to the length, and record the product of these two measurements in sq.% as the gland area. Neither of these methods take into account the growth and branching of the ducts which occur inside the perimeter of the gland.

A more informative technique is that of tracing or projecting the outline of the gland on to % squared graphic paper and counting the number of squares covered by the gland image. This technique is used in this study. It has also been the main measurement in the relative growth analyses of the mammary gland in the rhesus monkey and the adult female rat (Folley et al 1939; Cowie and Folley 1947; Cowie 1949; Silver 1953a and 1953b). It can also be used as a quantitative measurement of glandular development on its own (Hosi et al 1940; Richardson 1955). Its use is limited by the following:

Inaccuracy in the measuring technique (Nicol 1965). This is more marked in the very small glands measured such as the embryonic or postnatal rudimentary male glands. In this study the linear magnification of 316.2 X is used to diminish inaccuracy but this does not equalize errors in measurements of male/

male and female or prenatal and postnatal glands.

The measurement of area by this method depends on a staining reaction which gives a clear outline of the duct system. This is readily achieved in the postnatal glands dissected free from the skin but in the prenatal glands stained in the pelt, the clarity of staining is markedly diminished by background staining. The same difficulty arises in the staining of cultured glands where clarity of the gland outline is diminished by background staining particularly where the skin with the 16 day old gland rudiment in situ has the deeper connective tissues and anlage of the panniculus carnosus attached to its deep surface. In the cultures of this study, growth that occurred was completely organized (organotypic) with no diffuse peripheral spreading. This is largely due to culturing the organs on the surface of the cultivation medium (Moscona et al 1965).

The technique of preparing whole mounts, which includes mounting between glass slides, may lead to some degree of flattening of the ducts. This will affect the female gland with its extensive lumen more than the smaller male gland with its solid epithelial end-buds. The outline area of a shadowgraph of the female gland which is almost completely two dimensional if so flattened, will approach half of its complete external surface area. The outline area of a shadowgraph of a male gland however, will be close to a third of the complete external surface area in view of the rudimentary state of the gland with only a lumen in the main ducts and a larger proportion of the gland consisting of solid epithelial buds.

Accurate/

Accurate comparisons between the male and female glands with this method are therefore not possible.

Shrinkage will affect area measurements in the same way as duct length measurements.

The rate of increase in area is less than the rate of increase in duct length in the prenatal female gland, whereas in the male gland they are more similar. This is due to the rapid increase in the number of end-buds in the female gland which affects the duct length measurement progressively more than the area measurement. This explains why the regression slope in the female gland is less than that in the male gland in the relative growth analysis of prenatal development.

From the above, it would seem that the usefulness of area measurements as a means of comparison is limited to studies on glands from animals of the same sex and stage of development.

(e) Arborescence.

A method which compares the type of structure in the relatively unbranched gland of the untreated suckling animal, with that obtained in the more complex oestrogen-stimulated organ, was developed by Flux (1954) for the mouse mammary gland and adopted by Silver (1953a; 1953b) in her studies on the rat gland. It consists of the projection of an image of the gland on to squared paper at a magnification suitable for counting side-buds and duct-junctions. In small glands all the duct-junctions and buds were counted, but in those in which considerable growth and branching had occurred this was not practicable, and the number of ducts and buds was estimated by counting those present in a quarter of the total number of squares occupied by the/
the/

the projected gland. The squares analysed were selected by means of a table of random numbers. The total surface area had previously been determined, and it was therefore possible to reduce the above estimates of mammary arborescence to a figure representing the number of buds or duct-junctions per % square of gland. The principles and criticisms which apply to area measurements in general also apply to this lengthy procedure.

(ii) Analysis based on the histology of the mammary glands.

(a) Arborescence.

A method developed for the determination of the internal surface area of lung alveoli (Short 1950), has been applied to the study of the mammary gland of the goat (Cowie et al 1952; Richardson 1949), and of the rat (Silver 1953a; 1954).

This involves the use of a grid of lines of known total length which is imposed upon fields chosen at random from histological sections of the mammary gland. The number of intersections made by the grid lines with the epithelial lining is counted and used for the determination of the internal surface area. The application of this technique to any organ depends on the uniformity of the epithelial distribution or arrangement in the organ. In the mammary gland it cannot give a true over-estimation of arborescence at the nipple region where only primary and secondary ducts with a few side branches and buds are present and is also not applicable to very small undeveloped male mammary glands (Silver 1953a; 1953b).

(b) Mammary gland area measurements.

A method which measures the surface area of the glandular tree in mice from histological serial sections has been developed by/

by Squartini (1957). Having delimited a selected microscopic field by means of an ocular micrometer, the field is reproduced to scale by tracing the outline of the epithelial structures of the gland at a known magnification. The epithelium tracings are then cut out along their outlines and the pieces of paper weighed on a precision scale. By comparing the weight of the pieces with the weight of a square % of the same type of paper, the surface area occupied by the glandular epithelium can be calculated in square %. This laborious technique has several possible sources of inaccuracy and has little advantage over the other methods described earlier.

(c) Mitotic index and gland volume.

Very useful information can be obtained about the growth rate of the mammary glands by calculating the mitotic index in different parts of the mammary tree. This can then be compared with the mitotic index in the epidermis, as a tissue from which the mammary glands are derived and which therefore is a convenient standard, against which the mitotic activity of the gland can be tested (Balinsky 1950). Cellular proliferation is proportional to the growth rate only if the size of the cells does not change and if there is no loss of cells. Both these conditions apply at least approximately to the mammary rudiment in the prenatal mouse (Balinsky 1950). The main danger of mitotic index determinations lies in the evaluation of the statistical significance which can be attached to the figures obtained. Pasteels (1940) critically reviews this method and Balinsky (1950) discusses the precautions necessary for a quantitative analysis. A study on cellular proliferation by mitotic/

mitotic indices should be supplemented by studies on the growth of the gland as a whole. Balinsky (1950) obtains the volume of the glands in arbitrary units by drawing serial sections of the glands by means of a camera lucida and then measuring the surface of each section with a planimeter. The sum of these areas give a crude approximation of the gland volume. This method seems accurate but tedious and could be replaced by one of the other methods described.

(d) Cellular proliferation.

DNA determinations.

It is possible to show a direct relationship between the DNA content per dry weight of an organ, and the number of nuclei present in a given volume of that organ. Various methods for determining DNA have been developed in an attempt to use changes in the DNA content as a quantitative index of cellular proliferation and therefore of growth in the mammary glands of mice and other animals exposed to exogenous hormones (Kirkham and Turner 1953; Harkness and Harkness 1956; Griffith and Turner 1957; Damm and Turner 1957 and 1958; and Brookreson and Turner 1959).

Naito (1958) in a study on the guinea-pig mammary gland during lactation, points out that this technique assumes that the relation between the dry weight and the sectional area of the gland remains constant throughout different stages, and that the number of cells per microscopic field (obtained by multiplying the number of cells per modal alveolus by the number of alveoli per field), is statistically reliable. This technique however, indiscriminately measures the contribution of all the cellular/

cellular components of the mammary gland. The DNA derived from connective tissue, vascular and other elements could be significant particularly in the very active early mammary growth (Traurig and Morgan 1964). The technical difficulties involved and the inaccuracies which exist in this method (Nicoll 1965) make it unfavourable as a method for quantitative analysis of mammary gland development.

Autoradiography.

The technique of autoradiography with tritiated thymidine has also been used to measure the proliferative response of the adult spayed female mouse mammary epithelium to hormonal treatment (Traurig and Morgan 1964). This method is based on the fact that thymidine is a specific precursor for DNA synthesis by cells entering mitosis. Tritium bound to thymidine will not exchange with hydrogen in the surrounding medium, and the thymidine will not be replaced once it has been incorporated in DNA, unless this cell synthesizes more DNA preparatory to a further division. Dilution of the tracer in the tissue thus occurs during cellular proliferation (Leblond et al 1959; Amano et al 1959). As in the DNA determinations, this method needs specialized equipment and is not favourable as a routine method.

III. Sexual differentiation in the C57BL^t/H mouse.

From the microscopical and whole amount analysis in this study the characteristics of sexual differentiation are clearly established. They can be summarised as follows:-

- (i) Presence or absence of nipple formation.

The first clear sign of sexual differentiation occurs at the/

the 17th day of embryonic life with the separation of the male sprout from the epidermis. This separation is persistent for the period studied and is reflected throughout later development by the continued absence of nipples in the male. The female sprout begins to develop a nipple at the 18th day of embryonic life and retains it throughout further development.

(ii) Growth rate.

After the 17th day prenatally, the growth rate is considerably higher in the female gland than in the male, particularly during the remainder of prenatal development. This is clearly illustrated by:

- (a) The rate of increase of end-buds
- (b) The rate of increase in duct length
- (c) The rate of increase in gland area.

These two phenomena^(i, ii) were also described by Raynaud (1947a, b, c; 1949 a, b), Raynaud and Frilley (1949) and Raynaud and Raynaud (1956), and accepted as the characteristic signs of sexual differentiation in their series of experimental studies on the mammary gland of the XXX strain of mice.

(iii) Overall gland appearance.

The female gland rapidly develops into the typical "tree" of a future compound exocrine gland with secondary and tertiary ducts, whereas the male remains rudimentary in its appearance consisting of one or occasionally two main ducts with short epithelial end-buds only.

These three criteria can therefore be used to decide whether a gland in culture is following a male or female pattern of mammary gland development.

(IV)/

(IV) The relative growth analysis of normal development.

The limitations in area measurement techniques discussed earlier makes it clear that the value of a relative growth analysis by the method of Huxley (1932) is doubtful. In particular, the analysis of small glands such as the glands of male and female embryos or postnatal males are likely to give inaccurate results.

Useful information can be gained, however, when one considers and compares relative growth in glands which belong to the same sex and to the same period of development.

(i) Prenatal glands.

Both males and females grow allometrically (positively) between the ages of 18 days and 20 days of embryonic life. This agrees with the microscopic analysis of Balinsky (1950).

(ii) Postnatal glands.

(a) Females.

From birth to 21 days of age the slope of the regression line does not deviate significantly from the value of 1 required for isometry. This isometric growth in the female mouse mammary gland is in agreement with the findings of Cowie (1949) and Silver (1953a; 1953b) in the rat.

(b) Males.

From birth to 21 days of age the slope of the regression line lies well below the value of 1 and suggests that the male glands grow allometrically (negatively) during this phase.

(V) Comparison of left and right glands in male and female mice.

The observations on paired glands of male and female mice at/

at 21 days of age illustrated that the left and right glands in the same animal show very little deviation from each other in number of end-buds. Inspection of measurements of all the prenatal and postnatal glands confirms that this similarity in paired glands persist throughout the period of development studied.

The method of experimental approach is important and is in many ways responsible for the confusion which exists in the understanding of hormone action in experimental animals. Ablation of the endocrine glands the physiological balance of the body. Emergency endocrine mechanisms in the general body reaction may alter or obscure local observations in the mammary glands. The treatment of the last 40 years produces many contradictory findings.

The earliest interest is focused on the ovarian hormones as a result of the first successful effort to demonstrate the presence in the ovaries of a factor which causes growth of the uterus by Folio (1913). His ovarian extract injections into rabbits was followed by many studies with crude extracts until Doisy et al (1930) crystallized a pure estrogen from human menopausal urine. During this period, transplantation experiments also provide some information of ovarian action on the mammary glands. Autotransplants of mouse mammary glands (Stricker 1939) and functional ovarian grafts into young ovariectomized mice (Gardner 1935) are some of the early techniques employed. These methods supplemented with surgical ablation of the ovaries are the basic techniques to acquire knowledge about hormonal action on the mammary gland. Literature/

2. Experimental study.

The dependence of the mouse mammary gland on several hormone combinations to achieve growth and functional activity is well established. It is, however, difficult to evaluate the part played by any one factor in a living organism where many interactions are continuously taking place (Lasfargues and Murray 1959). The method of experimental approach is important and is in many ways responsible for the confusion which exists in the understanding of hormone action in experimental animals. Ablation of the endocrines upsets the physiological balance of the body. Emergency endocrine mechanisms in the general body reaction may alter or obscure local observations in the mammary glands. The research of the last 40 years produces many contradictory findings.

The earliest interest is focussed on the ovarian hormones as a result of the first successful effort to demonstrate the presence in the ovaries of a factor which causes growth of the mammae by Fellner (1913). His ovarian extract injections into rabbits are followed by many studies with crude extracts until Doisy et al (1930) crystallize a pure oestrogen from human pregnancy urine. During this period, transplantation experiments also provide some information of ovarian action on the mammary glands. Autotransplants of mouse mammary glands (Stricker 1929) and functional ovarian grafts into young castrated male mice (Gardner 1935) are some of the early techniques employed. These methods supplemented with surgical ablation of the ovaries are the basic techniques to acquire knowledge about hormonal action on the mammary gland. Abundant literature/

literature on the ovarian hormones is reviewed by Speert (1948) and Folley (1952).

The discovery that neoplastic transformation of mammary epithelium is influenced by a hormonal environment especially by oestrogens (Laccassagne 1932) stimulates Trentin and Turner (1948) and Elliot and Turner (1953) to carry out further investigations on oestrogens in order to clarify their role. They find that a combination of oestradiol and progesterone increase ductal and lobulo-alveolar formation in the adult female spayed and male castrate mouse mamma. An oestrogen/progesterone ratio of 1/1000 favours maximal expansion. Laccassagne and Chamorro (1939), Selye (1940), Leonard and Reece (1942) and later Trentin and Turner (1948) and Elliot and Turner (1953) demonstrate that ovarian hormones fail to produce this stimulation of mammary growth in adult rats and mice following hypophysectomy. This leads to the conclusion that oestrogens only have the function of physiological stimulation of the mammotropic hormones of the pituitary, and in combination with progesterone they act as synergists of the pituitary Furth and Clifton (1957), Nandi (1958). Ahren and Jacobsen (1957) having obtained growth of the mammary glands in the complete absence of pituitary hormones in hypophysectomized adult female spayed rats by means of daily injections of long acting insulin and an oestradiol-progesterone combination, question the growth promoting potential of the hypophysis and conclude that any hormone endowed with a powerful metabolic action can play a role in mammary gland growth. Dao (1962) however gives evidence that the mammo-

tropic/

tropic action of prolactin from a pituitary graft is direct and that its effect is independent of ovarian hormones,

Trentin and Turner (1948) also show that adrenalectomy results in rapid regression of mammary alveolar tissue. Oestrogens, which they find induce an extensive development of mammary glands in castrate male rats, produce no effect in castrate adrenalectomized animals. This suggests to them that the adrenals have a mediatory role in mammogenesis. Selye (1954) demonstrates the positive influences of the gluco-corticoids on rat mammary development and his findings are confirmed by Johnson and Meites (1955) and Faulkin and de'Ome (1958). Ahren and Jacobsen (1956, 1957) find that after hypophysectomy cortisone produces only an enlargement of the ducts without normal growth or differentiation in adult albino female spayed rats. The state of uncertainty which exists as a result of these many observations on different rat and mouse strains of different age and sex and under different experimental conditions is reflected by the attempt of Lyons et al (1958) to correlate all experimental data. The lack of complete understanding of the role of hormones in mammogenesis is demonstrated by their general postulate viz: "In the Long-Evans rat, at least 5 of the 6 well-identified anterior pituitary hormones (Mammothropin, Corticotropin, Somatotropin, Thyrotropin and the gonadotropins, follicle-stimulating hormone and interstitial cell stimulating hormone) play important parts in mammogenesis and lactogenesis. The thyroid and therefore Thyrotropin is unnecessary in mammogenesis but the possibility remains that the/

the mammary gland may utilize iodinated compounds independently of the thyroid".

Following the differentiation of mouse mammary gland rudiments in culture by Hardy (1950) and the review on the effects of carcinogens, hormones and vitamins in organ cultures by Lasnitzki (1958), Lasfargues and Murray (1959) accepting that organ culture methods may be a valuable method for analysing the endocrine control of mammatogenesis, point out that the ideal approach is to eliminate the organism altogether, and to transplant the mammary gland under study into an environment as nearly physiological as possible, and then to observe its reactions in the presence of specific factors. Lasfargues and Murray (1959) also accept the following conditions:

(a) The "histological environment of the gland is preserved while interrelationships with the organism are severed". Exactly what this means is not clear.

(b) "Since organ cultures are by definition short term cultures (12 days maximum in their study), commercial synthetic nutrients can supply the basic nutritive components necessary for the development of the explants".

(c) "In a system where all elements are known it is possible to determine with precision the effects produced by one variable into the medium". It is difficult to accept unconditionally this statement.

They (Lasfargues and Murray 1959) then proceed to investigate the specific action of the three main hormonal groups (Estradiol and progesterone, growth hormone and mammatropin, and cortisol) involved in mammary growth and lactation.

Culturing/

Culturing skin fragments from C57B mouse embryos of 10-15 days of age by the watch-glass method of Fell and Robison (1929) in Morgans 199 medium (Paul 1965) only, they obtain some differentiation of mammary epithelium but report that patches of necrosis in the "adipose and connective tissues" occur after 12 days in culture. The "modicum" of differentiation is apparently not sufficient to allow recognition of the sex of the explanted glands. They also find that oestradiol and progesterone together inhibit the growth of the mammary epithelium, while oestrogens alone stimulate adipose tissue formation and progesterone alone suppresses connective tissue development. Growth hormone (L2722 BS, bovine origin) with mammotropin (L2651BS, of ovine origin) promote active growth of the mammary epithelium, with mammotropin acting specifically on adipose tissue. They state that cortisol conditions the gland for secretion and adduce as evidence of this the "distension" of the lumen of the ducts to form thin walls of active secretory cells associated with an increase of the surrounding adipose tissue.

They conclude that: "The growth and function of the mammary gland is regulated by a succession of events triggered in time by specific hormones for specific purposes. Only one hormone appears to be primarily and directly concerned with the growth of the mammary epithelium: growth hormone. The ovarian hormones by their action on the stroma prepare the gland for growth, but mammotropin and cortisol condition its secretory function".

As a result of the work of Lasfargues and Murray (1959), numerous/

numerous studies in organ culture have been carried out over the last 7 years.

Sidman (1956), Elias (1959), Trowell (1959) and Rivera and Bern (1961) find that insulin as the only protein component of a synthetic medium can maintain the organotypic structure of normal experimental animal adult tissues in vitro for several days. Moretti and de'Ome (1962) show that this dependency on insulin is lost with neoplastic change. Bern and Rivera (1960) and Prop (1961a) find that hormones in particular prolactin, cannot exert their action in absence of insulin. Ketterer et al (1957) find that in the organism the presence of insulin is necessary for growth hormone to become effective. This is supported by the work of Hay (1958) on the effect of growth hormone and insulin on limb-bone rudiments of chick-embryos in culture. Elias (1959), Prop (1961a) and Rivera (1964 a and b) state that insulin and aldosterone should be included in all hormone-supplemented media to ensure survival of explanted glands. Koziorowska (1962) however states that insulin may actively suppress oestradiol action. Lasnitzki (1965) reviews the various findings and concludes that insulin is a growth promoting agent which effects proliferation in organ culture directly and stimulates glycogen synthesis and lipogenesis. It also selectively increases RNA synthesis. Which of these effects are primary is not known.

Early work on the role of hormones in the aetiology of mammary cancer by Laccassagne (1932), Loeb and Kirtz (1934) and Silberberg and Silberberg (1949) supplemented by more recent in vivo experiments by Ranadive (1952, 1953), stimulated
Ranadive/

Ranadive and Chapekar (1964) to carry out in vitro studies to test in vivo observations. They investigate three strains of mice varying from a highly susceptible tumour strain (the C3H (Jax) strain (Snell et al 1960)), to a low susceptible tumour strain (the ICRC strain (Ranadive and Kanekar 1963)), and a cancer resistant Swiss strain (Waravdekar and Ranadive 1962). Their results indicate that as in the in vivo experiments prolactin and progesterone in the presence of insulin and hydrocortisone have a direct effect on the development of the mouse mammary glands but the response is differential with a strain specificity retained even in the in vitro conditions.

Rivera (1964b) demonstrates that the addition of 5% hypophysectomized male Long-Evans rat serum increases the response to hormones of whole mammary gland explants from virgin female BALB/cCrGl mice in culture in a synthetic medium of known constitution. Because of the ineffectiveness of the serum alone Rivera (1964b) believes that serum may provide essential growth factors whose activity in mammary differentiation requires the presence of hormones in the culture medium. The finding of regression of whole mammary gland explants of glands from 6 weeks old BDF or CBA mice by Prop (1961a) and from adult mice by Koziorowska (1962) in (serum containing) media, supports view. Rivera (1964b) reports that the growing extremities are more sensitive than the larger ducts and show regression first. Other observations by Elias (1962) and Rivera (1963) on cultures of isolated portions of immature mouse glands, lend support to this finding of a difference in hormone dependence in different parts/

parts of the mammary gland.

It is clear from the above account that the problems of mammosgenesis and of the many factors on which it depends are by no means solved. The use of different culture techniques and different media both in constitution and concentrations applied to experimental animals of different strains, age and sex has made the problems more complex rather than simpler.

Standardisation of all these factors is obviously needed to bring order to the situation. A culture technique which achieves mammary gland development with sexual differentiation in a way which resembles normal mammosgenesis as closely as possible in the same strain of mouse in vivo, would provide the ideal baseline from which controlled experimental studies can be carried out. The work described in this study has shown that the development of such a technique may be possible.

The following fundamental observations have been made in this study.

I. Mammary gland rudiments from male and female embryos aged 16 days explanted for 5 days in Trowell's T8 medium with maternal serum and either male or female embryo extract under controlled conditions undergo organized (organotypic) growth. The rate of growth falls far short of that of control glands in vivo. This behaviour of the mammary gland in culture is similar to that of the mouse salivary gland rudiments in culture (Borghese 1950).

II. It is clear that both male and female gland rudiments make an attempt to simulate their typical in vivo pattern of differential sexual development if cultured in the appropriate medium i.e. the 16th day embryonic male gland develops into a typical male gland when cultured in Trowell's T8 medium + maternal/

maternal serum + male embryo extract and its female counterpart develops into a typical female gland when cultured in Trowell's T8 + maternal serum + female embryo extract. This behaviour in both sexes is not altered by the presence or absence of adjacent gland rudiments of the opposite sex in the same medium.

III. If cultured in the medium containing the extract of embryos of the opposite sex, the glands follow to a certain degree the opposite sexual differential pattern i.e. male rudiments follow the female pattern and female rudiments follow the male pattern. This behaviour also is not altered by the presence or absence of adjacent gland rudiments of the opposite sex in the same culture.

IV. Under all the above conditions the female rudiments grow better than the male rudiments.

V. Mammary gland rudiments from male and female embryos aged 16 days, explanted for 5 days in a solution consisting of Trowell's T8 medium only, undergo no growth or differentiation, although they maintain a normal histological appearance. The following conclusions can be drawn from all the observations:

Qualitative development in vitro:

The similar qualitative differentiation of male and female explants in the same medium clearly illustrates the dependence of both gland rudiments for their normal differential sexual development on factors present in the medium. These results correspond closely to the series of in vivo experiments (of Raynaud and his colleagues) on the XXX strain of embryonic mice:

(1) Injections of small doses of oestrogens over a period of/

of time into a pregnant mouse will induce a female mammo-genesis in male embryos but one which does not attain that found in female embryos of the same litter (Raynaud 1947c and 1949a).

(ii) Injections of androgens over the same period of time, into a pregnant mouse will induce a male mammogenesis in the female embryos (Raynaud 1947a; 1947b; 1949a; 1949b).

(iii) Destruction of embryonic testes by irradiation results in a female mammary histogenesis whereas destruction of embryonic ovaries does not affect final histogenesis (Raynaud and Frilley 1947 and 1949).

Experimental work on postnatal mice also gives a similar result. Injections of oestrogenic hormones directly into weanling male Swiss Schneider mice, produce feminization in the mammary glandular pattern (Hadfield and Young¹⁹⁵⁶), and transplantation of whole mammary glands from 3-50 weeks old female mice of either the C57BL/6J or C3H/HeJ strains into 3-26 weeks old F1 females of crosses between C57BL/6J and C3H/HeJ mice, results in the development of a functioning feminine gland even to the extent of secretory function during lactation of the host. (Thompson 1963). Transplantation of an adult male gland to a female host results in a similar feminine change in the gland system although there is no establishment of a nipple which, during lactation, renders the transplanted gland cystic with secretion in view of its discontinuity with the epidermis. (Thompson 1965).

Quantitative development in vitro:

The different quantitative development of male and female explants/

explants in the same medium clearly illustrates the presence of an inherent sexual differential response of the embryonic rudiments which, however, does not become evident without the addition of maternal serum or embryonic extracts. This view agrees with the conclusions of Hardy (1950), although her views are based on findings in cultures of mouse mammary rudiments in adult cock plasma and chick embryo extract, and with that of Lasfargues and Murray (1959) who cultured their organs in Morgan's 199 and Parker's 858 media. As a result of their work, these authors (Hardy 1950; Lasfargues and Murray 1959) state that the embryonic mammary gland development is not entirely dependent on hormonal action. This is clearly not a possible conclusion in the case of the explants of Hardy (1950) where hormones could have been introduced into her cultivation medium in the cock plasma or chick embryo extract.

Absence of development in vitro.

The lack of growth of any of the gland rudiments in Trowell's T8 medium alone, while maintaining a normal organotypic structure is contrary to the findings of Lasfargues and Murray (1959) who obtained penetration of 10-15 day mouse embryo abdominal epithelium into the adjacent connective tissue where it formed tubes. Their explants were in Morgan's 199 and Parker's 858 media as stated before and also other non-specified synthetic media which they do not discuss in detail. As mentioned above, growth of the mammary rudiments obtained by Hardy (1950) was not in a wholly defined medium without hormones. Neither Lasfargues and Murray (1959) nor Hardy (1950) attempted to distinguish sex in their experiments.

It/

It is obviously necessary to investigate this difference of growth potential in Trowell's T8 medium and the media used by Lasfargues and Murray (1959). Techniques, length of time in culture, and strain differences added to the different media used may all have a bearing on this finding.

Further possible research projects on the basic method developed in this study are numerous.

An attempt should be made to improve on the results obtained. In particular, growth which is quantitatively more like that found in vivo would give a wider scope for experimental work. Possible improvements may be achieved by investigating the following aspects of organ cultures of the mammary glands in particular and culture techniques in general.

(a) The calculation of the optimum concentrations of embryo extracts and maternal serum for each day should be carried out and applied (Gaillard 1935).

(b) An attempt should be made to establish whether extracts of only parts of embryos would not produce a better growth response in view of the findings of Durel et al (1965) that cultures of epitheliomata grew better in a substrate of mesonephros or liver extract whereas gonadal extract is less favourable and lung and skin extracts are definitely unfavourable. They conclude that certain embryonic tissues are more favourable to organ culture growth than others.

(c) Vitamin A has been shown to have a growth promoting effect on cells in culture by Baker (1936), Vollmar (1939) and Lasnitzki (1955 and 1958). The effects of this vitamin on organ culture is still the subject of extensive research (Fell and/

and Rinaldini (1965) and as yet not enough is known about its effect.

(d) Investigations of possible growth autoregulating factors both local and systemic, which may influence mammary gland growth in culture e.g. inflammatory exudates from canine pleurae has been shown to produce a proliferation response in the mammary glands of nonpregnant rabbits by Menkin (1955). Paschkis (1958) and Nicoll (1965) review work on this problem.

(e) General advances in organ culture techniques which depend on the discovery of the factors which maintain cell life in vivo should be applied to this technique (Moscona et al 1965).

Once a standardized technique is established, controlled experimental studies of individual and combined hormonal supplements to the medium can be carried out using the normal development and sexual differentiation of male and female glands in vitro as controls.

The normal development of the third pair of mammary glands is similar to that of the first pair. The mammary glands of male and female mice of the C57BL/6 strain, were examined from their first appearance in the embryo up to the age of 30 days postnatally. Serial sections and whole mounts of mammary glands were made and a quantitative analysis of the number of mammary glands, the total duct length and the ductal area, was carried out.

Using an organ culture method, male and female 16 day embryonic mammary glands were grown in a medium containing male or female embryonic extracts.

The results obtained were:

1. In both sexes the first appearance of the mammary glands occurs during the 14th to 15th day of embryonic life and mammary glands in identical up to the 16th day postnatally.

SUMMARY AND CONCLUSIONS.

2. The mammary glands of male and female mice develop separately and remain separate throughout the period covered by this study. No nipple formation takes place and the glands appear rudimentary in structure.

3. The female mammary glands remain attached to the epiblasts, forming a nipple and undergo rapid growth and differentiation during the period studied.

4. The right and left sides of the gland pairs in both sexes are very similar throughout the development studied.

5. Male and female embryonic mammary glands cultivated in the presence of male embryonic extract, develop along a male developmental pattern and in the presence of female embryonic extract, develop along a female developmental pattern. In all cases the female explants grow better than the male explants.

These results were compared with other studies on mammary gland development.

The normal development of the third pair of thoracic mammary glands in male and female mice of the C57BLa^t/H strain, has been studied from its first appearance in the embryo up to the age of 21 days postnatally. Serial sections and whole mount preparations were used and a quantitative analysis of the number of end-buds, the total duct length and the outline gland area, was carried out.

Using an organ culture method, male and female 16 day embryonic gland rudiments were grown in a medium containing male or female embryonic extracts.

The results obtained were:

1. In both sexes the first appearance of the mammary gland occurs during the 11th to 12th day of embryonic life and mammo-genesis is identical up to the 16th day prenatally.

2. The male gland separates from the epidermis at 17 days prenatally and remains separate throughout the period covered by this study. No nipple formation takes place and the gland system remains rudimentary in its structure.

3. The female gland remains attached to the epidermis, forms a nipple and undergoes rapid growth and differentiation during the period studied.

4. The right and left sides of the gland pairs in both sexes are very similar throughout the development studied.

5. Male and female embryonic glands cultivated in the presence of male embryonic extract, develop along a male mammo-genetic pattern and in the presence of female embryonic extract, develop along a female mammo-genetic pattern. In all cases the female explants grow better than the male explants.

These results were compared with other studies on mammary gland development.

A	Arteria albuginea.
B	Primary bud.
C	Secondary cap.
C	Capillary.
D	Primary lactiferous duct.
d	Secondary duct.
E	Epithelial outgrowth.
e	End-bud.
G	Germinal epithelium.
G	Germ cells.
H	Hair follicle.
M	Smooth muscle fibres.
N	Necrosis.
N	Nipple.

ABBREVIATIONS. cell nucleus.

O	Ovary.
P	Primary pit.
P	Panniculus carnosus.
S	Primary sprout.
s	Secondary sprout.
T	Tertiary duct.
t	Spermatic tubule.
V	Endothelial lined space.
X	Testis.

A	Tunica albuginea.
B	Primary bud.
C	Mammary cap.
c	Capillary.
D	Primary lactiferous duct.
d	Secondary duct.
E	Epithelial downgrowth.
e	End-bud.
G	Germinal epithelium.
g	Germ cells.
H	Hair follicle.
M	Smooth muscle fibres.
m	Mitosis.
N	Nipple.
n	Myo-epithelial cell nucleus.
O	Ovary.
P	Mammary pit.
p	Panniculus carnosus.
S	Primary sprout.
s	Secondary sprout.
T	Tertiary duct.
t	Spermatic tubule.
V	Endothelial lined space.
X	Testis.

A C K N O W L E D G E M E N T S.

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ALLEN, W. (1934). EFFECT OF VIBRATIONS ON THE
GROWTH OF THE EMBRYO.

Ann. Entomol. Soc. Amer., 27: 121.

ALLEN, W. (1935). THE EFFECT OF VIBRATIONS ON
THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

ALLEN, W. (1936). THE EFFECT OF VIBRATIONS ON
THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

Ann. Entomol. Soc. Amer., 29: 193.

ALLEN, W. and JAMESON, D. (1937). THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

Ann. Entomol. Soc. Amer., 30: 254.

ALLEN, W. (1938). THE EFFECT OF VIBRATIONS ON
THE GROWTH OF THE EMBRYO.

ALLEN, W., JAMESON, D., and DOBBS, E. (1939). THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO AND THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO.

ALLEN, W., JAMESON, D., DOBBS, E., and DOBBS, E. (1940). THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO AND THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO.

ALLEN, W., JAMESON, D., DOBBS, E., and DOBBS, E. (1941). THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO AND THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO.

BIBLIOGRAPHY.

ALLEN, W., JAMESON, D., and DOBBS, E. (1942). THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO AND THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO.

ALLEN, W., and JAMESON, D. (1943). THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

ALLEN, W., JAMESON, D., and DOBBS, E. (1944). THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO AND THE EFFECT
OF VIBRATIONS ON THE GROWTH OF THE EMBRYO.

ALLEN, W. (1945). THE EFFECT OF VIBRATIONS ON
THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

ALLEN, W. (1946). THE EFFECT OF VIBRATIONS ON
THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

ALLEN, W. (1947). THE EFFECT OF VIBRATIONS ON
THE GROWTH OF THE EMBRYO AND THE EFFECT OF VIBRATIONS
ON THE GROWTH OF THE EMBRYO.

ABERLE, S.B.D. (1934a) GROWTH OF MAMMARY GLAND IN THE RHESUS MONKEY.

Proc. Soc. Exp. Biol., N.Y., 32: 249.

ABERLE, S.B.D. (1934b) PROLIFERATION OF EPITHELIUM OF NIPPLE OF THE RAT AND GUINEA-PIG DURING THE OESTRUS CYCLE.

Proc. Soc. Exp. Biol., N.Y., 32: 245.

AHREN, K. and JACOBSON, D. (1956). MAMMARY GROWTH IN HYPOPHYSECTOMIZED RATS INJECTED WITH OVARIAN HORMONES AND INSULIN.

Acta Physiol. Scand., 37: 190.

AHREN, K. and JACOBSON, D. (1957). THE ACTION OF CORTISONE ON THE MAMMARY GLANDS OF RATS UNDER VARIOUS STATES OF HORMONAL IMBALANCE.

Acta. Physiol. Scand., 40: 254.

ALLEN, E. (1922). THE OESTRUS CYCLE IN THE MOUSE.

Am. J. Anat., 30: 297.

ALLEN, E., DANFORTH, C.H., and DOISY, E.A. (1939) (eds). SEX AND INTERNAL SECRETIONS, 2nd. ed.

Baltimore, Williams and Wilkins.

ALLEN, E., FRANCIS, B.F., ROBERTSON, L.L., COLGATE, C.F., JOHNSTON, C.G., DOISY, E.A., KOUNTZ, W.B. and GIBSON, H.V. (1924). THE HORMONE OF THE OVARIAN FOLLICLE, ITS

LOCALIZATION AND ACTION IN TEST ANIMALS, AND ADDITIONAL POINTS BEARING UPON THE INTERNAL SECTION OF THE OVARY.

Am. J. Anat., 34: 133

AMANO, M., MESSIER, B., and LEBLOND, C.P. (1959). SPECIFICITY OF LABELLED THYMIDINE AS A DEOXYRIBO-NUCLEIC ACID PRECURSOR IN RADIOAUTOGRAPHY.

J. Histochem. Cytochem., 7: 153.

ANCEL, P., and BOUIN, P. (1911). SUR LE DETERMINISME DU DEVELOPPEMENT DE LA GLANDE MAMMAIRE AU COURS DE LA GESTATION.

Journ. Physiol. et Pathol., 13: 31.

ARGYRIS, B.F., and ARGYRIS, T.S. (1960). MAMMARY DUCT STIMULATION BY SUBCUTANEOUS EHRLICH ASCITES TUMOUR TRANSPLANTS.

Cancer Research 20: 325.

BABKIN, B.P. (1944). SECRETORY MECHANISMS OF THE DIGESTIVE GLANDS.

New York: Paul B. Hoeber, 695.

BAKER, L.E. (1936). EFFECT OF VITAMIN A ON PROLIFERATION OF FIBROBLASTS.

Proc. Soc. Exp. Biol., N.Y., 33: 124

BALINSKY, B.I. (1950). ON THE PRENATAL GROWTH OF THE MAMMARY GLAND RUDIMENT IN THE MOUSE.

J. of Anat., 84: 227.

- BANG, F., VEILLISTO, I. and LIBERT, R. (1956a).
I. STUDY OF NORMAL AND MALIGNANT CELLS FROM THE MAMMARY GLAND OF MICE.
Bull. J. Hopkins Hosp., 98: 255.
- BANG, F., ANDERVONT, H.B. and VEILLISTO, I. (1956b).
II. AN E/M STUDY OF SPONTANEOUS AND INDUCED MAMMARY TUMOURS OF MICE.
Bull. J. Hopkins Hosp., 98: 287.
- BASCH, K. (1903). DIE PHYSIOLOGIE DER MILCHABSONDERUNG.
Ergebnisse der Physiologie Vol. 2, 1: 325.
- BERN, H.A. and RIVERA, E.M. (1960). EFFECT OF HORMONES ON ORGAN CULTURES OF MOUSE MAMMARY TISSUES.
Proc. Am. Ass. Canc. Res., 3: 94.
- BERTKAU, F. (1907). EIN BEITRAG ZUR ANATOMIE UND PHYSIOLOGIE DER MILCHDRUSE.
Anat. Anz., 30: 161.
- BITTNER, J.J. (1939). BREAST CANCER IN BREEDING AND VIRGIN "A" AND "B" STOCK FEMALE MICE AND THEIR HYBRIDS.
U.S. Pub. Health. Rep., 54: 1113.
- BLAIR, S.M., BLAIR, P.B., and DAANE, T.A. (1957). DIFFERENCES IN THE MAMMARY RESPONSE TO ESTRONE AND PROGESTERONE IN CASTRATE MALE MICE OF SEVERAL STRAINS AND HYBRIDS.
Endocrinology., 61: 643.
- BLANDAU, R.J., BOLING, J.L., and YOUNG, W.C. (1941). THE LENGTH OF HEAT IN THE ALBINO RAT AS DETERMINED BY THE COPULATORY RESPONSE.
Anat. Rec., 79: 453.
- BONNET, R. (1892). DIE MAMMARORGANE UND LICHTER DER ONTOGENIE UND PHYLOGENIE.
Ergebn. d. Anat. und Entwicklungsgeschichte., 2: 604.
- BORGHESE, E. (1950). THE DEVELOPMENT IN VITRO OF THE SUBMANDIBULAR AND SUBLINGUAL GLANDS OF MUS MUSCULUS.
J. Anat., 84: 287.
- BRAIN, E.B. (1949). A PRELIMINARY INVESTIGATION INTO THE SHRINKAGE OF THE ORAL TISSUES DUE TO EMBEDDING IN PARAFFIN WAX.
Brit. dent. J., 87: 32.
- BRAMBELL, F.W.R. (1927). THE DEVELOPMENT AND MORPHOLOGY OF THE GONADS OF THE MOUSE. PART I THE MORPHOGENESIS OF THE INDIFFERENT GONAD AND OF THE OVARY.
Proc. Roy. Soc. B., 101: 316.
- BRAMBELL, F.W.R. and PARKES, A.S. (1927). THE NORMAL OVARIAN CYCLE IN RELATION TO OESTRUS PRODUCTION.
Quart. J. Exp. Physiol., 18: 185.
- BRESSLAU, E. (1920). THE MAMMARY APPARATUS OF THE MAMMALIA.
London. Methuen and Co. Ltd.

- BROOKRESON, A.D. and TURNER, C.W. (1959). NORMAL GROWTH OF MAMMARY GLAND IN PREGNANT AND LACTATING MICE. *Proc. Soc. Exp. Biol. Med.*, 102: 744.
- BUCHSBAUM, R. and KUNZ, J.A. (1954). EFFECTS OF CERTAIN STIMULANTS AND DEPRESSANTS ON INDIVIDUAL FIBROBLASTS IN PERFUSION CHAMBER. *Ann. N.Y. Acad. Sci.*, 58: 1303.
- CARLETON, H.M. and DRURY, R.A.B. (1957). (eds). HISTOLOGICAL TECHNIQUE. 3rd ed. Oxford Univ. Press, Lond.
- CARREL, A. and EBELING, A.H. (1923). ACTION OF SERUM ON FIBROBLASTS IN VITRO. *Journ. of Exp. Med.*, 37: 759.
- CHARLTON, H.H. (1917). THE FATE OF THE UNFERTILIZED EGG IN THE WHITE MOUSE. *Biol. Bull.*, 33: 321.
- CHEN, J.M. (1954). THE CULTIVATION IN FLUID MEDIUM OF ORGANISED LIVER, PANCREAS AND OTHER TISSUES OF FOETAL RATS. *Exp. Cell Research.*, 7: 518.
- CHRISTIANSEN, G.S., DANES, B., ALLEN, L. and LEINFELDER, P.J. (1953). A CULTURE CHAMBER FOR THE CONTINUOUS BIOCHEMICAL AND MORPHOLOGICAL STUDY OF LIVING CELLS IN TISSUE CULTURE. *Exp. Cell Research.*, 5: 10.
- CLAUBERG, C. (1931). GENITALCYCLUS UND SCHWANGERSCHAFT BEI DER WEISSEN MAUS. (ANATOMISCHE STUDIEN AN OVARIUM, UTERUS UND SCHEIDE) DAUER DES GENITALCYCLUS. *Arch. Gynäk.*, 147: 549.
- CLOUDMAN, A.M. (1941). SPONTANEOUS NEOPLASMS IN MICE. In: *Biology of the Laboratory Mouse* Snell, G.D. (ed) The Blakiston Co., Philadelphia.
- COLE, H.A. (1934). THE MAMMARY GLAND OF THE MOUSE DURING THE OESTRUS CYCLE, PREGNANCY AND LACTATION. *Proc. Roy. Soc. B.*, 114: 136.
- CONSTABLE, F.L. and MOFFAT, M.A.J. (1958). A GLASS TISSUE CULTURE CHAMBER FOR USE IN TIME-LAPSE CINEMATOGRAPHY. *J. Clin. Pathol.*, 11: 455.
- COWIE, A.T. (1949). THE RELATIVE GROWTH OF THE MAMMARY GLAND IN NORMAL, GONADECTOMIZED AND ADRENALECTOMIZED RATS. *J. Endocrin.*, 6: 145.
- COWIE, A.T. and FOLLEY, S.J. (1947). THE ROLE OF THE ADRENAL CORTEX IN MAMMARY DEVELOPMENT AND ITS RELATION TO THE MAMMOGENIC ACTION OF THE ANTERIOR PITUITARY. *Endocrinology.*, 40: 274.

COWIE, A.T., FOLLEY, S.J., MALPRESS, F.H., and RICHARDSON, K.C. (1952). STUDIES ON THE HORMONAL INDUCTION OF MAMMARY GROWTH AND LACTATION IN THE GOAT.

J. Endocrin., 8: 64.

CRUICKSHANK, C.N.D., COOPER, J.R. and CONRAN, M.B. (1959). A NEW TISSUE CULTURE CHAMBER.

Exp. Cell Research., 16: 695.

CULLINGS, C.F.A. (1957). HANDBOOK OF HISTOPATHOLOGICAL TECHNIQUE.

Butterworth, Lond.

DAMM, H.C. and TURNER, C.W. (1957). DESOXYRIBOSE NUCLEIC ACID AS INDEX OF MAMMARY GLAND GROWTH OF MICE.

Proc. Soc. Exp. Biol., N.Y., 25: 466.

DAMM, H.C. and TURNER, C.W. (1958). EFFECTS OF HORMONE DOSE AND LENGTH OF INJECTION ON MOUSE MAMMARY GLAND GROWTH.

Proc. Soc. Exp. Biol., 98 (1): 192.

DANFORTH, C.H. (1916). THE USE OF EARLY DEVELOPMENTAL STAGES IN THE MOUSE FOR CLASS WORK IN EMBRYOLOGY.

Anat. Rec., 10: 355.

DANIEL, J.F. (1910). OBSERVATIONS ON THE PERIOD OF GESTATION IN WHITE MICE.

J. Exp. Zool., 2: 865.

DAO, T.L. (1962). MAMMARY CARCINOGENESIS BY 3-METHYLCHOLANTHRENE. IV. EFFECT OF PITUITARY HOMOGRAFT ON MAMMARY-GLAND GROWTH AND TUMORIGENESIS.

J. Nat. Cancer. Inst., 29: 107.

DAO, T.L., TANAKA, Y and GAWLAK, D. (1964). TUMOR INDUCTION IN TRANSPLANTED MAMMARY GLANDS IN RATS.

J. Nat. Cancer. Inst., 32: 1259

DAWSON, E.K. (1934). A HISTOLOGICAL STUDY OF THE NORMAL MAMMA IN RELATION TO TUMOUR GROWTH. I. EARLY DEVELOPMENT TO MATURITY.

Edinb. med. J., 41: 653.

DICK, D.A.T. (1955). AN EASILY MADE TISSUE CULTURE PERFUSION CHAMBER.

Quart. J. Microscop. Sci., 96: 363.

DOISY, E.A., VELER, C.D. and THAYER, S. (1930). PREPARATION OF THE CRYSTALLINE OVARIAN HORMONE FROM THE URINE OF PREGNANT WOMEN.

Jour. Biol. Chem., 86: 449.

DU BOIS, J. (1941). DETAILS TECHNIQUES SUR LE PRELEVEMENT L'ETALEMENT ET LE MONTAGE EN TOTALITE DES GLANDES MAMMAIRES DE LA SOURIS.

C.R. de la Soc. de Biol., 135: 52.

DU BOIS, J. (1944). METHODE D'EVALIATION QUANTITATIVE DU DEVELOPPEMENT DE LA GLANDE MAMMAIRE CHEZ LA SOURIS TRAITEE PAR DES CORPS OESTROGENES.

C.R. de la Soc. de Biol., 138: 149.

DUREL, J., WOLFF, E. and WOLFF, E. (1965). CULTURE ORGANOTYPIQUE D'UN EPITHELIOMA HUMAIN EN PRESENCE DE DIFFERENTS ORGANES EMBRYONNAIRES.

Arch. Anat. Micr. Morph. Exp., 54: 659.

ECKHARD, C. (1855). QUOTED BY LANE-CLAYPON AND STARLING (1906).

EDWARDS, J.E. (1950). MASSON'S TRICHROME CONNECTIVE-TISSUE STAIN FOOT AND GOLDNER MODIFICATION.

McClung's Handbook of Microscopical Technique p. 249.

Ed. McClung Jones, R. 3rd Edition. Paul B. Hoeber, Inc., Medical Book Dept. of Harper and Brothers, N.Y.

ELIAS, J.J. (1959). EFFECT OF INSULIN AND CORTISOL ON ORGAN CULTURES OF ADULT MOUSE MAMMARY GLAND.

Proc. Soc. Exp. Biol. and Med., 101: 500

ELIAS, J.J. (1962). RESPONSE OF MOUSE MAMMARY DUCT END-BUDS TO INSULIN IN ORGAN CULTURE.

Exp. Cell. Res., 27: 601.

ELLIOTT, J.R., and TURNER, C.W. (1953). THE MAMMARY GLAND SPREADING FACTOR.

Res. Bull. Mo. Agric. Exp. Sta., 537: 52.

EMERY, F.E. and SCHWABE, E.L. (1936). THE VAGINAL SMEARS OF RATS AS INFLUENCED BY FREQUENT EXAMINATIONS.

Anat. Rec., 64: 147.

FARRIS, E.J. (1941). APPARATUS FOR RECORDING CYCLICAL ACTIVITY IN THE RAT.

Anat. Rec., 81: 357.

FAULKIN, L.J. and DE OME, K.B. (1958). THE EFFECT OF ESTRADIOL AND CORTISOL ON THE TRANSPLANTABILITY AND SUBSEQUENT FATE OF NORMAL HYPERPLASTIC AND TUMEROUS MAMMARY TISSUE OF C₃H MICE.

Cancer Research., 18: 51.

FELL (1965). PERSONAL COMMUNICATION.

FELL, H.B., and ROBISON, R. (1929). THE GROWTH, DEVELOPMENT AND PHOSPHATASE ACTIVITY OF EMBRYONIC AVIAN FEMORA AND LIMB BUDS CULTIVATED IN VITRO.

Biochem. J., 23: 767.

FELL, H.B., and RINALDINI, L.M. (1965). THE EFFECTS OF VITAMINS A AND C ON CELLS AND TISSUES IN CULTURE.

IN: CELLS AND TISSUES IN CULTURE Vol. I (Ed) E.N. Willmer. Acad. Press, London and N.Y.

FELLNER, O.O. (1913). EXPERIMENTELLE UNTERSUCHUNGEN UBER DIE WIRKUNG VON GEWEBSEXTRAKTEN AUS DER PLAZENTA UND DEN WEIBLICHEN SEXUALORGANEN AUF DAS GENITALE.

Arch. f. Gynäk., 100: 641.

FISCHER, A. and PARKER, R.C. (1929). PROLIFERATION UND DIFFERENZIERUNG.

Arch. f. Exp. Zellf., 8: 297.

FLUX, D.S. (1952). QUOTED BY SILVER (1954).

FOLLEY, S.J. (1947). THE NERVOUS SYSTEM AND LACTATION.
Brit. Med. Bull., 5: 142.

FOLLEY, S.J. (1949). NEURO-HORMONAL MECHANISMS IN LACTATION.
J. Endocrinol., 6: 16.

FOLLEY, S.J. (1952). LACTATION. IN: MARSHALL'S PHYSIOLOGY
OF REPRODUCTION. (A.S. Parker, ed), Vol. II pp 525.
Longmans, Green, London, New York and Toronto.

FOLLEY, S.J. GÜTHKELCH, A.N. and ZUCKERMAN, S. (1939).
THE MAMMARY GLAND OF THE RHESUS MONKEY UNDER NORMAL AND
EXPERIMENTAL CONDITIONS.
Proc. Roy. Soc. B., 126: 469.

FURTH, J., and CLIFTON, K.H. (1957). EXPERIMENTAL OBSERVATIONS
ON MAMMOTROPES AND THE MAMMARY GLAND. In: ENDOCRINE
ASPECTS OF BREAST CANCER.
(A.R. Currie, ed) pp. 276 Livingstone, Edinburgh and London.

GAILLARD, P.J. (1935). DEVELOPMENTAL CHANGES IN THE COMPOSITION
OF THE BODY FLUIDS IN RELATION TO GROWTH AND DIFFERENTIATION
OF TISSUE CULTURES.
Protoplasma., 23: 174.

GARDNER, W.U. (1935). THE EFFECT OF OVARIAN HORMONES AND
OVARIAN GRAFTS UPON THE MAMMARY GLANDS OF MALE MICE.
Endocrinology., 19: 656.

GARDNER, W.U. and STRONG, L.C. (1935). THE NORMAL DEVELOPMENT
OF THE MAMMARY GLANDS OF VIRGIN FEMALE MICE OF TEN STRAINS
VARYING IN SUSCEPTIBILITY TO SPONTANEOUS NEOPLASMS.
Am. J. Cancer., 25: 282.

GEGENBAUER, C. (1876). ZUR GENAUEREN KENNTNIS DER ZITZENDER
SAUGETHIERE.
Morph. Jahrb., 1: 266.

GERARD, R.W. (1931). OXYGEN DIFFUSION INTO CELLS.
Biol. Bull., 60: 245.

GIBSON, L.M. (1930). A COMPARATIVE STUDY OF THE LIFE HISTORY
OF THE FEMALE MAMMARY GLAND IN TWO STRAINS OF ALBINO MICE.
J. Cancer Res., 14: 570.

GOLTZ and EWALD (1896). QUOTED BY LANE-CLAYPON AND STARLING (19

GRIFFITH, D.R. and TURNER, C.W. (1957). DESOXYRIBONUCLEIC ACID
(DNA) CONTENT OF MAMMARY GLAND DURING PREGNANCY AND LACTATION.
Proc. Soc. Exp. Biol., N.Y. 25: 347.

GRUNBERG, H. (1943). THE DEVELOPMENT OF SOME EXTERNAL FEATURES
IN MOUSE EMBRYOS.
J. Hered., 34: 88.

- HADFIELD, G. (1958). THE APPLICATION OF PHYSIOLOGICAL PRINCIPLES TO HORMONE-DEPENDENT BREAST CANCER. *Annals of the Roy. Col. of Surgeons of England.*, 22: 73.
- HADFIELD, G. and YOUNG, J.S. (1956). THE MAMMOTROPHIC POTENCY OF HUMAN URINE. *Brit. J. of Cancer.*, 10: 145.
- HAMPERL, H. (1939). UBER DIE MYOTHELIUM (MYO-EPITHELIALEN ELEMENTE) DER BRUSTDRUSE. *Virchows Arch.*, 305: 171.
- HARDY, M.H. (1949). THE DEVELOPMENT OF MOUSE HAIR IN VITRO WITH SOME OBSERVATIONS ON PIGMENTATION. *J. of Anat.*, 83: 365.
- HARDY, M.H. (1950). THE DEVELOPMENT IN VITRO OF THE MAMMARY GLANDS OF THE MOUSE. *J. of Anat.*, 84: 388.
- HARKNESS, M.L.R., and HARKNESS, R.D. (1956). THE EFFECT OF PREGNANCY AND LACTATION ON THE COLLAGEN CONTENT OF THE MAMMARY GLAND OF THE RAT. *J. Physiol.*, 132: 476.
- HARRIS, H. (1955). SOME QUANTITATIVE STUDIES ON MULTIPLICATION OF CONNECTIVE TISSUE CELLS IN VITRO. *Brit. J. Exper. Path.*, 36: 115.
- HARRISON, R.G. (1907). OBSERVATIONS ON THE LIVING DEVELOPING NERVE FIBRE. *Proc. Soc. Exp. Biol.*, N.Y. 4: 140.
- HAY, M.F. (1958). THE EFFECT OF GROWTH HORMONE AND INSULIN ON LIMB-BONE RUDIMENTS OF THE EMBRYONIC CHICK CULTIVATED IN VITRO. *J. Physiol.*, 144: 490.
- HEAPE, W. (1900). THE SEXUAL SEASON. *Quart. Jour. Mic. Sc.*, 44: 1.
- HENNEBERG, B. (1900). DIE ERSTE ENTWICKELUNG DER MAMMARORGANE BEI DER RATTE. *Anat. Hefte.*, 13: 1.
- HEUVERSWYN, J. van., FOLLEY, S.J. and GARDNER, W.U. (1939). MAMMARY GROWTH IN MALE MICE RECEIVING ANDROGENS, OESTROGENS AND DESOXYCORTICOSTERONE ACETATE. *Proc. Soc. Exp. Biol and Med.*, 41: 389.
- HIRSCHLAND, L. (1899). BEITRAGE ZUR ERSTEN ENTWICKELUNG DER MAMMARORGANE BEIM MENSCHEN. *Anat. Hefte.*, 11: 221.
- HOLLMANN, K.H. (1959). L'ULTRASTRUCTURE DE LA GLANDE MAMMAIRE NORMALE DE LA SOURIS EN LACTATION (ETUDE AU MICROSCOPE ELECTRONIQUE). *J. Ultrastructure Res.*, 2: 423.

HOSHINO, K. (1962). MORPHOGENESIS AND GROWTH POTENTIALITY OF MAMMARY GLANDS IN MICE. I. TRANSPLANTABILITY AND GROWTH POTENTIALITY OF MAMMARY TISSUE OF VIRGIN MICE. J. Nat. Canc. Inst., 29: 835.

HOSI, T., SAKISAKA, T. and KAWAHARA, Y. (1940). QUOTED BY COWIE (1948). Jap. J. Vet. Sci., 2: 412.

HOWARD, J.G. (1964). PERSONAL COMMUNICATION.

HU, F.N., HOLMES, S.G., POMERAT, C., LIVINGOOD, C.S. and McCONNELL, K.P. (1951). METHOD FOR STUDYING EFFECT OF P32 ON LIVING ADULT HUMAN EPIDERMAL CELLS IN PERFUSION CHAMBER. Texas Rep. Biol. and Med., 2: 739.

HUSS, M. (1873). BEITRAGE ZUR ENTWICKELUNGSGESCHICHTE DER MILCHDRUSE BEIM MENSCHEN UND BEI WIEDERKAUERN. Jena Zeitsch. F. Med. u. Nature., 7: 176.

HUXLEY, J.S. (1932). (ed) PROBLEMS OF RELATIVE GROWTH London.

JACKSON, C.M. (1912). ON THE RECOGNITION OF SEX THROUGH EXTERNAL CHARACTERS IN THE YOUNG RAT. Biol. Bull., 23: 171.

JOHNSON, R.M., and MEITES, J. (1955). EFFECTS OF CORTISONE, HYDROCORTISONE AND ACTH ON MAMMARY GLAND GROWTH AND PITUITARY PROLACTIN CONTENT OF RATS. Proc. Soc. Exp. Biol. Med., 89: 455.

KALLIUS, E. (1897). EIN FALL VON MILCHLEISTE BEI EINEM MENSCHLICHEN EMBRYO. Anat. Hefte., 8: 153.

KETTERER, B. RANDLE, P.J. and YOUNG, F.O. (1957). THE PITUITARY GROWTH HORMONE AND METABOLIC PROCESSES. Ergebn. Physiol., 49: 127.

KIRKHAM, W.B. (1907). THE MATURATION OF THE MOUSE EGG. Biol. Bull., 12 (No. 4): 259.

KIRKHAM, W.B. (1916). THE PROLONGED GESTATION PERIOD IN SUCKLING MICE. Anat. Rec., 11: 31.

KIRKHAM, W.R. and TURNER, C.W. (1953). NUCLEIC ACIDS OF THE MAMMARY GLANDS OF RATS. Proc. Soc. Exp. Biol. and Med., 83: 123.

KOCH, F.C. (1937). THE MALE SEX HORMONES. Physiol. Rev., 17: 153.

KOLLIKER, T. (1879). BEITRAGE ZUR KENNTNIS DER BRUSTDRUSE Wurzburger Verhandlungen, N.F., 14.

- KOZIOROWSKA, J. (1962). THE INFLUENCE OF OVARIAN HORMONES AND INSULIN ON THE MOUSE MAMMARY GLANDS CULTIVATED IN VITRO. *Acta Med. Pol.*, 3: 237.
- KREBBS, H.A. and JOHNSON, W.A. (1948). CELL METABOLISM. *Tabul. Biol.*, (Hague) 19: 100.
- LACCASSAGNE, A. (1932). APPARITION DE CANCERS DE LA MAMELLE CHEZ LA SOURIS MALE SOUMISE A DES INJECTIONS DES FOLLICULINE. *C. R. acad. sci.*, 195: 630
- LACCASSAGNE, A. and CHAMORRO, A (1939). CONSEQUENCES DE L'HYPOPHYSECTOMIE CHEZ DES SOURIS SUJETTES AU CARCINOME MAMMAIRE, TRAITEMENTS PAR HORMONE ESTROGENE. *C.R. de la soc. de biol.*, 131: 1077.
- LANE-CLAYPON, J.E. and STARLING E.H. (1906). AN EXPERIMENTAL ENQUIRY INTO THE FACTORS WHICH DETERMINE THE GROWTH AND ACTIVITY OF THE MAMMARY GLANDS. *Proc. Roy. Soc.*, B 77: 505.
- LANGER, C. (1871). MILCHDRUSE. Stricker's Handbuch Der Lebre von den Geweben des Menschen und der Thiere (Leipzig: W. Engelmann) 1: 627. English Translation: Henry Power (1872) Manual of Human and Comparative Histology.
- LANGER, E. and HUHN, S. (1958). DER SUBMIKROSKOPISCHE BAU DER MYOEPIHELZELLE. *Z. Zellforsch.*, 47: 507.
- LASFARGUES, E.Y. and MURRAY, M.R. (1959). HORMONAL INFLUENCES ON THE DIFFERENTIATION AND GROWTH OF EMBRYONIC MOUSE MAMMARY GLANDS IN ORGAN CULTURE. *Develop. Biol.*, 1: 413.
- LASNITZKI, I. (1955). THE EFFECT OF EXCESS VITAMIN A ON MITOSIS IN CHICK HEART FIBROBLASTS IN VITRO. *Exp. Cell Res.*, 8, 121.
- LASNITZKI, I. (1958). THE EFFECT OF CARCINOGENS, HORMONES AND VITAMINS ON ORGAN CULTURES. *Intern. Rev. Cytol.*, 7: 79.
- LASNITZKI, I. (1965). THE ACTION OF HORMONES ON CELL AND ORGAN CULTURES. In: CELLS AND TISSUES IN CULTURE. Vol. I. Ed. Willmer, E.N. Acad. Press, London, New York.
- LEBLOND, C.P., MESSIER, B. and KOPRIWA, B. (1959). THYMIDINE - H³ AS A TOOL FOR THE INVESTIGATION OF THE RENEWAL OF CELL POPULATIONS. *Lab. Invest.*, 8: 296.

- LENFERS, P. (1907). ZUR HISTOLOGIE DER MILCHDRUSE DES RINDES.
Z. Fleisch - U. Milchhyg., 17: 340, 383, 424.
- LEONARD, S.L., and REESE, R.P. (1942). FAILURE OF STEROID
HORMONES TO INDUCE MAMMARY GROWTH IN HYPOPHYSECTOMISED RATS.
Endocrinology., 30: 32.
- LEWIS, W.H. and WRIGHT, E.S. (1935). ON THE EARLY DEVELOP-
MENT OF THE MOUSE EGG.
Carnegie Instn. Wash. Pub., 459: 113.
- LINZELL, J.L. (1952). THE SILVER STAINING OF MYOEPIHELIAL
CELLS, PARTICULARLY IN THE MAMMARY GLAND, AND THEIR RELATION
TO THE EJECTION OF MILK.
J. Anat., 86: 49.
- LOEB, L. (1897). "UBER DIE ENTSTEHUNG VON BINDEGEWEBE,
LEUCOCYTEN UND ROTEN BLUTKORPERCHEN AUS EPITHEL UND UBER
EINE METHODE, ISOLIERTE GEWEBESTEILE ZU ZUCHTEN".
M. Stern and Co., Chicago.
- LOEB, L., and KIRTZ, M.M. (1934). THE EFFECTS OF TRANSPLANTS
OF ANTERIOR LOBES OF THE HYPOPHYSES ON THE GROWTH OF THE
MAMMARY GLAND AND ON THE DEVELOPMENT OF MAMMARY GLAND
CARCINO IN VARIOUS STRAINS OF MICE.
Am. J. Cancer., 36: 56.
- LONG, J.A., and EVANS, H.M. (1922). THE OESTRUS CYCLE IN
THE RAT AND ITS ASSOCIATED PHENOMENA.
Mem. Univ. Cal., 6: 1.
- LONG, J.A. and MARK, E.L. (1911). THE MATURATION OF THE EGG
OF THE MOUSE.
Carnegie Instn. Wash. Pub., 142: -1.
- LUDWIG, (1851). QUOTED BY LANGER (1871).
- LYONS, W.R., LI, C.H. and JOHNSON, R.E. (1958). THE HORMONAL
CONTROL OF MAMMARY GROWTH AND LACTATION.
Recent Progr. in Hormone Research., 14: 219.
- MACKANASS, G.B. (1952). ACTION OF DRUGS ON INTRACELLULAR
TUBERCLE BACILLI.
J. Path. and Bact., 64: 429.
- McKENNA, J.M. and STEVENS, K.M. (1957). THE EARLY PHASE OF THE
ANTIBODY RESPONSE.
J. Immunol., 78: 311.
- MAU, J.C.H. de., RIJSSSEL, Th. G. van (1961). ELECTRON
MICROSCOPY OF TISSUE OF THE MAMMARY GLANDS AND TUMOURS
IN OLD MICE WITH SPECIAL REFERENCE TO MITOCHONDRIAL SIZE.
J. Nat. Cancer Inst., 26: 919.
- MAXIMOV, A. (1925). TISSUE CULTURES OF YOUNG MAMMALIAN EMBRYOS.
Contr. Embryol. Carneg. Instn., 16: 47.

MEEH, K. (1879). OBERFLACHENMESSUNGEN DES MENSCHLICHEN KORPERS.
Z. Biol., 15: 425.

MENKIN, V. (1955). EFFECT ON BREAST TISSUE OF A PROLIFERATIVE
FACTOR PRESENT IN INFLAMMATORY EXUDATES.
Proc. Amm. Assoc. Cancer Research., 2: 34.

MERTON, H. (1938). STUDIES ON REPRODUCTION IN THE ALBINO
MOUSE I. THE PERIOD OF GESTATION AND THE TIME OF
PARTURITION.
Proc. Roy. Soc. Edinburgh., 58: 80.

MERTON, H. (1939). STUDIES ON REPRODUCTION IN THE ALBINO
MOUSE. III. THE DURATION OF LIFE OF SPERMATOZOA IN THE
FEMALE REPRODUCTIVE TRACT.
Proc. Roy. Soc. Edinburgh., 59: 207.

MIRSKAIA, L., and CREW, F.A.E. (1930). MATURITY IN THE
FEMALE MOUSE.
Proc. Roy. Soc. Edinburgh., 50: 179.

MISE, S. (1961). EXPERIMENTAL STUDIES ON MICRORADIOAUTO-
GRAPHY USING RADIOACTIVE PHOSPHORUS (P32) IN NEOPLASTIC
DISEASE OF THE BREAST IN MICE.
Arch. Jap. Chir., 287: 2782.

MIYAWAKI, H. (1965). HISTOCHEMISTRY AND ELECTRON MICROSCOPY
OF IRON CONTAINING GRANULES, LYSOSOMES, AND LIPOFUSCIN IN
MOUSE MAMMARY GLANDS.
J. Nat. Canc. Inst., 34: 601.

MOON, R.C., GRIFFITH, D.R. and TURNER, C.W. (1959). NORMAL AND
EXPERIMENTAL GROWTH OF RAT MAMMARY GLAND.
Proc. Soc. Exp. Biol., 101/4: 788.

MORETTI, R.L., and DE OME, K.B. (1962). EFFECT OF INSULIN ON
GLUCOSE UPTAKE BY NORMAL AND NEOPLASTIC MOUSE MAMMARY TISSUES
IN ORGAN CULTURE.
J. Nat. Canc. Inst., 29: 321.

MOSCONA, A., TROWELL, O.A., and WILLMER, E.N. (1965). METHODS.
In: CELLS AND TISSUES IN CULTURE Vol. I. (ed) E.N. Willmer.
Acad. Press London and New York.

MYERS, J.A. (1917). STUDIES ON THE MAMMARY GLAND II. THE
FOETAL DEVELOPMENT OF THE MAMMARY GLAND IN THE FEMALE ALBINO RAT.
Am. J. Anat., 22: 195.

NAGAI, J., YAMADA, J., YOSHIDA, M., CHIKAMUNE, T., and NAITO, M.
(1957). VARIATION OF THE MAMMARY GLANDS RESPONSE OF INBRED
FEMALE MICE TREATED WITH ESTROGEN.
Endocrinol. Japan., 4: 12.

NAITO, M. (1958). HISTOLOGICAL CHANGES IN THE MAMMARY GLAND OF
GUINEA-PIGS DURING LACTATION.
J. of Dairy Res., 25: 392.

NANDI, S. (1958). ROLE OF SOMATOTROPIN IN MAMMOGENESIS AND LACTOGENESIS IN C₃H/HE CRGL MICE. Science., 128: 772.

NANDI, S. (1961). DIFFERENTIAL RESPONSIVENESS OF A AND C3H MOUSE MAMMARY TISSUES TO SOMATOTROPIC-CONTAINING HORMONE COMBINATIONS. Proc. Soc. Exp. Biol. Med., 108: 1.

NICHOLAS, J.S. (1942). EXPERIMENTAL METHODS AND RAT EMBRYOS. In: THE RAT IN LABORATORY INVESTIGATION. (Griffith, J.Q. and Farris, E.J. Eds) page 50. J.B. Lippincott Co. Philadelphia, Montreal, London.

NICOLL, C.S. (1965). GROWTH AUTOREGULATION AND THE MAMMARY GLAND. J. Nat. Canc Inst., 34: 131.

OWEN, R. (1868). (Ed.) ON THE ANATOMY OF VERTEBRATES VOL.III MAMMALS. London. Longmans, Green and Co.

PAPANICOLAOU, G.N. (1933). THE SEXUAL CYCLE IN THE HUMAN FEMALE AS REVEALED BY VAGINAL SMEARS. Am. J. Anat. (Suppl.), 52: 519.

PARKES, A.S. (1928). THE LENGTH OF THE OESTRUS CYCLE IN THE UNMATED NORMAL MOUSE: RECORDS OF 1000 CYCLES. Brit. J. Exp. Biol., 2: 371.

PARKES, A.S. (1929). THE INTERNAL SECRETION OF THE OVARY. London, Longmans.

PASTEELS, J. (1940). UN APERCU COMPARATIF DE LA GASTRULATION CHEZ LES CHORDES. Biol. Rev., 15: 53.

PASCHKIS, K.E. (1958). GROWTH PROMOTING FACTORS IN TISSUES: A REVIEW. Cancer Res., 18: 981.

PAUL, J. (1965) (Ed). CELL AND TISSUE CULTURE. E. and S. Livingstone Ltd., Edinburgh and London.

PETERS, A. (1958). STAINING OF NERVOUS TISSUE BY PROTEIN-SILVER TECHNIQUES. Stain Technol., 33: 47.

PINCUS, G. (1936). (Ed) THE EGGS OF MAMMALS. The Macmillan Co., New York.

PINCUS, G. and THIMANN, K.V. (1948). (Eds) THE HORMONES, Vol. 1 New York, Acad Press.

PINCUS, G. and THIMANN, K.V. (1950). (Eds) THE HORMONES, Vol. 2 New York, Acad. Press.

PINCUS, G. and THIMANN, K.V. (1955). (Eds) THE HORMONES, Vol. 3. New York, Acad. Press.

PIPKIN, A.C. and MACK, A.D. (1958). A NEW TISSUE CULTURE FLASK WITH DEMOUNTABLE BOTTOM.
Exp. Cell Res., 14: 219.

PITELKA, D.R., BERN, H.A., NANDI, S., and DE OME, K.B. (1964). ON THE SIGNIFICANCE OF VIRUS-LIKE PARTICLES IN MAMMARY TISSUES OF C3HF MICE.
J. Nat. Canc. Inst., 33: 867.

PROP, F.J.A. (1959). ORGAN CULTURES OF TOTAL MAMMARY GLANDS OF THE MOUSE.
Nature., 184: 379.

PROP, F.J.A. (1960). DEVELOPMENT OF ALVEOLI IN ORGAN CULTURES OF TOTAL MAMMARY GLANDS OF SIX WEEKS OLD VIRGIN MICE.
Exp. Cell Res., 20: 256.

PROP, F.J.A. (1961a). EFFECTS OF HORMONES ON MOUSE MAMMARY GLANDS IN VITRO. ANALYSIS OF THE FACTORS THAT CAUSE LOBULO-ALVEOLAR DEVELOPMENT.
Path. et Biol., 9: 640.

PROP, F.J.A. (1961b). ACTIONS MORPHOGENETIQUES D'HORMONES SUR LA GLANDE MAMMAIRE IN VITRO.
Ann. Endocrin. (Paris), 22: 826.

PROP, F.J.A. (1961c). HORMONES AND MAMMARY TISSUE IN VITRO.
Acta Physiol. Pharmacol. Neerlandica., 10: 305.

PROP, F.J.A. (1963). HYPOPHYSEAL HORMONES AND MAMMARY GLANDS IN ORGAN CULTURES.
Acta Physiol. Pharmacol. Neerlandica., 12: 172.

PULVERTAFT, R.J.V., HAYNES, J.A. and GROVES, J.T. (1956). "PERSPEX" SLIDES FOR ROLLER CULTURE OF HUMAN CELLS.
Exp. Cell Res., 11: 99.

RANADIVE, K.J. (1945). COMPARATIVE MORPHOLOGY OF NORMAL MAMMARY GLANDS OF FOUR STRAINS OF MICE, VARYING IN THEIR SUSCEPTIBILITY TO BREAST CANCER.
Proc. Ind. Acad. Sci., 22: B, 18.

RANADIVE, K.J. (1952). HORMONAL FACTOR IN BREAST CARCINOMA.
Indian J. med. Sci., 6: 892.

RANADIVE, K.J. (1953). RELATIVE IMPORTANCE OF HEREDITY, HORMONES AND MILK-BORNE TUMOUR AGENT IN INDUCING MAMMARY CARCINOMA IN MICE.
Indian J. med. Sci., 7: 545.

RANADIVE, K.J. and CHAPEKAR, T.N. (1964). IN VITRO STUDIES ON MOUSE MAMMARY GLAND RESPONSE TO HORMONAL TREATMENT.
Brit. J. Canc., 18: 308.

RANADIVE, K.J. and KANEKAR, S.A. (1963). BIOLOGICAL STUDIES OF THE NEW ALBINO MOUSE INBRED AT THE INDIAN CANCER RESEARCH CENTRE.

Indian J. med. Res., 51: 1005.

RAYNAUD, A. (1947a). EFFET DES INJECTIONS D'HORMONES SEXUELLES A LA SOURIS GRAVIDE, SUR LE DEVELOPPEMENT DES EBAUCHES DE LA GLANDE MAMMAIRE DES EMBRYONS. 1. Action des substances androgenes.

Ann. D'Endocr., 8 No. 3: 248.

RAYNAUD, A. (1947b). ACTION DES HORMONES SEXUELLES SUR LE DEVELOPPEMENT EMBRYONNAIRE DE LA GLANDE MAMMAIRE DE LA SOURIS.

Acad. Sciences., 225: 150.

RAYNAUD, A. (1947c). EFFET DES INJECTIONS D'HORMONES SEXUELLES A LA SOURIS GRAVIDE, SUR LE DEVELOPPEMENT DE EBAUCHES DE LA GLANDE MAMMAIRE DES EMBRYONS.

II. Actions de fortes de substances oestrogenes.

Ann. D'Endocr., 8 No. 4: 318.

RAYNAUD, A. (1947d). OBSERVATIONS SUR LE DEVELOPPEMENT NORMAL DES EBAUCHES DE LA GLANDE MAMMAIRE DES FOETUS MALES ET FEMELLES DE SOURIS.

Ann. D'Endocr., 8: 349.

RAYNAUD, A. (1949a). EFFET, SUR LE DEVELOPPEMENT DE LA GLANDE MAMMAIRE DES EMBRYONS DE SOURIS, DES HORMONES SEXUELLES INJECTEES A LA SOURIS EN GESTATION.

Bull. Soc. Zool. France., LXXIV: 153.

RAYNAUD, A. (1949b). NOUVELLES OBSERVATIONS SUR L'APPAREIL MAMMAIRE DES FOETUS DE SOURIS. Provenant de meres ayant reçu des injections de testosterone pendant la gestation.

Ann. D'Endocr., 10 No. 1: 54.

RAYNAUD, A. and FRILLEY, M. (1947). ETAT DE DEVELOPPEMENT DES EBAUCHES MAMMAIRES ET DU CORDON VAGINAL CHEZ LES FOETUS MALES ET FEMELLES DE SOURIS, DONT LES EBAUCHES DES GLANDES GENITALES ONT ETE DETRUITES PAR UNE IRRADIATION AU MOYEN DES RAYONS X, A L'AGE DE TREIZE JOURS.

C.R. Acad. Sciences., 225: 1380.

RAYNAUD, A. and FRILLEY, M. (1949). DEVELOPPEMENT EMBRYONNAIRE DE LA GLANDE MAMMAIRE DE LA SOURIS, APRES DESTRUCTION, AU MOYEN DES RAYONS X, DES GLANDES GENITALES DE L'EMBRYON.

Bull. Soc. Zool. France., LXXIV, 156.

RAYNAUD, A. and RAYNAUD, J. (1956). LA PRODUCTION EXPERIMENTALE DE MALFORMATIONS MAMMAIRES CHEZ LES FOETUS DE SOURIS, PAR L'ACTION DES HORMONES SEXUELLES.

Ann. De L'institut Pasteur (Paris), 90: 39.

REIN, G. (1881-2). UNTERSUCHUNGEN UBER DIE EMBRYONALE ENTWICKELUNGSGESCHICHTE DER MILCHDRUSE.

Archiv. Fur mikr. Anatomie., 20: 21, 431, 678.

- RETTERER, E. and LELIEVRE, A. (1911). STRUCTURE COMPARE DE LA GLANDE MAMMAIRE A L'ETAT NORMAL ET PATHOLOGIQUE. J. Anat. Paris., 47: 101.
- RIBBERT (1898). QUOTED BY LANE-CLAYPON AND STARLING (1906).
- RICHARDSON, F.L. (1951). FURTHER STUDIES ON THE MAMMARY GLAND DEVELOPMENT IN MALE MICE AT NINE YEARS OF AGE. Anat. Rec., 111: 669.
- RICHARDSON, F.L. (1953). THE MAMMARY GLAND DEVELOPMENT IN NORMAL AND CASTRATE MALE MICE AT NINE WEEKS OF AGE. Anat. Rec., 117: 449.
- RICHARDSON, F.L. (1955). THE RELATIVE GROWTH OF THE MAMMARY GLANDS IN NORMAL OVARECTOMIZED AND ADRENALECTOMIZED FEMALE MICE. Anat. Rec., 123: 279.
- RICHARDSON, F.L. and CLOUDMAN, A.M. (1947). THE MAMMARY GLAND DEVELOPMENT IN MALE MICE AT NINE WEEKS OF AGE. Anat. Rec., 97: 223.
- RICHARDSON, K.C. (1947). SOME STRUCTURAL FEATURES OF THE MAMMARY TISSUES. B. Med. Bull., 2: 123.
- RICHARDSON, K.C. (1949). CONTRACTILE TISSUES IN THE MAMMARY GLAND, WITH SPECIAL REFERENCE TO MYOEPIHELIIUM IN THE GOAT. Proc. Roy. Soc. B., 136: 30.
- RICHTER, K.M., CLOUD, S. and WOODWARD, N.W. Jr. (1956). STUDIES ON THE MAINTENANCE OF CERTAIN ADULT, INTACT BAT ORGANS IN CULTURE. Anat. Record., 125: 576.
- RICHTER, K.M., CLOUD, S. and RITCHESON, B.R. (1957). THE CULTIVATION OF INTACT THYROID AND PARATHYROID GLANDS OF THE ADULT BAT. Anat. Record., 128: 605.
- RICHTER, K.M., RITCHESON, B.R. and CLOUD, S. (1957). STUDIES ON THE ADULT BAT ADRENAL GLAND CULTURED "IN VITRO". Anat. Record., 128: 607.
- RICHTER, K.M. and WOODWARD, N.W. Jr. (1955). A VERSATILE TYPE OF PERFUSION CHAMBER FOR LONG-TERM MAINTENANCE AND DIRECT MICROSCOPIC OBSERVATION OF TISSUES IN CULTURE. Exp. Cell Research., 9: 585.
- RIVERA, E.M. (1963). HORMONAL REQUIREMENTS FOR SURVIVAL AND GROWTH OF MOUSE PRIMARY MAMMARY DUCTS IN ORGAN CULTURE. Proc. Soc. Exp. Biol. (N.Y.), 114: 735.

RIVERA, E.M. (1964a). DIFFERENTIAL RESPONSIVENESS TO HORMONES OF C3H AND A. MOUSE MAMMARY TISSUE IN ORGAN CULTURE. Endocrinology., 74: 853.

RIVERA, E.M. (1964b). MAINTENANCE AND DEVELOPMENT OF WHOLE MAMMARY GLANDS OF MICE IN ORGAN CULTURE. J. Endocrin., 30: 33.

RIVERA, E.M., and BERN, H.A. (1961). INFLUENCE OF INSULIN OF MAINTENANCE AND SECRETORY STIMULATION OF MOUSE MAMMARY TISSUES BY HORMONES IN ORGAN CULTURE. Endocrinology., 69: 340.

ROSE, G.G. (1954). SEPARABLE AND MULTIPURPOSE TISSUE CULTURE CHAMBER. Texas Rep. Biol. and Med., 12: 1704.

ROUTH (1897). QUOTED BY LANE-CLAYTON AND STARLING (1906).

SABATINI, D.D., BENSCH, K. and BARNETT, R.J. (1963). THE PRESERVATION OF CELLULAR ULTRASTRUCTURE AND ENZYMATIC ACTIVITY BY ALDEHYDE FIXATION. J. Cell Biol., 17: 19.

SCHAEFFER, B.M. (1956). THE CULTURE OF ORGANS FROM THE EMBRYONIC CHICK ON CELLULOSE-ACETATE FABRIC. Exp. Cell Research., 11: 244.

SCHICKELE, G. (1899). QUOTED BY TURNER AND GOMEZ (1933).

SCHULTZE, O. (1892). UEBER DIE ERSTE ANLAGE DES MILCHDRUSENAPPARATES. Anat. anz., 7: 265.

SEAMAN, A.R. (1956). THE IN VITRO CULTIVATION OF THE PROSTATE GLAND OF THE ADULT MOUSE IN ALKALINE FLUID MEDIUM. Exp. Cell Research., 11: 283.

SEAMAN, A.R. and STAHL, S. (1956). THE UPTAKE OF I¹³¹ BY THE THYROID GLAND OF THE ADULT MOUSE AFTER IN VITRO CULTIVATION. Exp. Cell Research., 11: 220.

SELYE, H. (1940). EFFECT OF CHRONIC PROGESTERONE OVERDOSAGE ON THE FEMALE ACCESSORY SEX ORGANS OF NORMAL OVARECTOMIZED RATS. Anat. Rec., 78: 253.

SELYE, H. (1954). THE EFFECT OF CORTISOL UPON THE MAMMARY GLANDS. Acta Endocrinol., 17: 394.

SHIMKIN, M.B., WYMAN, R.S. and ANDERVONT, H.B. (1946). MAMMARY TUMOURS IN MICE FOLLOWING TRANSPLANTATION OF MAMMARY TISSUE. J. Nat. Canc. Inst., 7: 77.

SHORT, R.H.D. (1950). ALVEOLAR EPITHELIUM IN RELATION TO GROWTH OF THE LUNG. Philos. Trans. B., 235: 35.

- SIDMAN, R.L. (1956). THE DIRECT EFFECT OF INSULIN ON ORGAN CULTURES OF BROWN FAT.
Anat. Rec., 124: 723.
- SILBERBERG, R., and SILBERBERG, M. (1949). MAMMARY CANCER IN CASTRATE MALE MICE RECEIVING OVARIAN AND HYPOPHYSEAL GRAFTS AT VARIOUS AGES.
Proc. Soc. Exp. Biol. and Med., 70: 510.
- SILVER, M. (1953a). THE QUANTITATIVE ANALYSIS OF THE ROLE OF OESTROGEN IN MAMMARY DEVELOPMENT IN THE RAT.
J. Endocrin., 10: 17.
- SILVER, M. (1953b). THE ONSET OF ALLOMETRIC MAMMARY GROWTH IN THE FEMALE HOODED NORWAY RAT.
J. Endocrin., 10: 35.
- SILVER, M. (1954). THE ROLE OF FOLIC ACID IN HORMONALLY-INDUCED TISSUE GROWTH.
J. Endocrin., 10: 95.
- SNELL, G.D. (1941). (Ed) BIOLOGY OF THE LABORATORY MOUSE. The Blakiston Company, Philadelphia.
- SNELL, G.D. FEKETE, E., HUMMEL, K.P. and LAW, L.W. (1940). THE RELATION OF MATING, OVULATION AND THE ESTRUS SMEAR IN THE HOUSE MOUSE TO TIME OF DAY.
Anat. Rec., 76: 39.
- SNELL, G.D., STAATS, J., LYON, M.F., DUNN, L.C., GRUNBERG, H., HERTWIG, P., HESTON, W.E. (1960). (The Committee on Standardized Genetic Nomenclature for mice). STANDARDIZED NOMENCLATURE FOR INBRED STRAINS OF MICE SECOND LISTING.
Cancer Research., 20: 145.
- SOBOTTA, J. (1895). DIE BEDRUCHTUNG UND FURCHUNG DES EIES DER MAUS.
Archiv. F. mikr. Anat., 45: 15.
- SØRENSEN, S.P.L. (1912). UBER DIE MESSUNG UND BEDEUTUNG DER WASSENSTROFFIONENKONZENTRATION BEI BIOLOGISCHEN PROZESSEN.
Ergebn. Physiol., 12: 393.
- SPEERT, H. (1948). THE NORMAL AND EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND OF THE RHESUS MONKEY, WITH SOME PATHOLOGICAL CORRELATIONS.
Contr. Embryol. Carneg. Instn., 32 (208): 11.
- SQUARTINI, F. (1957). A NEW METHOD FOR THE STUDY OF THE MAMMARY GLAND OF THE MOUSE.
Cancer., 10: 179.
- STEVENS, K.M. and MCKENNA, J.M. (1957). ANTIBODY PRODUCTION IN A COMPLETELY IN VITRO SYSTEM.
Nature., 179: 870.

STOCKARD, C.R., and PAPANICOLAOU, G.N. (1917). THE EXISTENCE OF A TYPICAL OESTRUS CYCLE IN THE GUINEA-PIG - WITH A STUDY OF ITS HISTOLOGICAL AND PHYSIOLOGICAL CHANGES.

Am. J. Anat., 22: 225.

STRAHL, H. (1898). DIE ERSTE ENTWICKELUNG DER MAMMARORGANE BEIM MENSCHEN.

Anat. Anz., 14: 236.

STRICKER, P. (1929). TRANSPLANTATIONS DE GLANDES MAMMAIRES DANS L'OREILLE DE LA LAPINE IMPUBERE. EVOLUTION DES TRANSPLANTS SOUS L'INFLUENCE DU CORPS JAUNE ET DE LA GESTATION. C.R. Soc. Biol. Paris., 102: 1076.

THOMPSON, J.S. (1963). TRANSPLANTATION OF WHOLE MAMMARY GLANDS IN MICE.

Transplantation., 1: 526.

THOMPSON, J.S. (1965). TRANSPLANTATION OF WHOLE MAMMARY GLANDS FROM MALE TO FEMALE MICE.

Anat. Rec., 151: 425.

TOGARI, C. (1927). ON THE OVULATION OF THE MOUSE.

Nagoya J. Med. Sc. Japan., 2: 17.

TRAURIG, H.H. and MORGAN, C.F. (1964). AUTORADIOGRAPHIC STUDIES OF THE EPITHELIUM OF MAMMARY GLAND AS INFLUENCED BY OVARIAN HORMONES.

Proc. Soc. Exp. Biol and Med., 115: 1076.

TRENTIN, J.J. and TURNER, C.W. (1948). THE EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND WITH SPECIAL REFERENCE TO THE INTERACTION OF THE PITUITARY AND OVARIAN HORMONES.

Res. Bull. Mo. Agric. Exp. Sta., 418: 48.

TROWELL, O.A. (1949). CULTURE OF LYMPHOID TISSUE IN VITRO.

J. Physiol., 110: 4P.

TROWELL, O.A. (1952). THE CULTURE OF LYMPH NODES IN VITRO.

Exp. Cell Research., 3: 79.

TROWELL, O.A. (1954). A MODIFIED TECHNIQUE FOR ORGAN CULTURE IN VITRO.

Exp. Cell Research., 6: 246.

TROWELL, O.A. (1955). THE CULTURE OF LYMPHNODES IN SYNTHETIC MEDIA.

Exp. Cell Research., 9: 258.

TROWELL, O.A. (1959). THE CULTURE OF MATURE ORGANS IN A SYNTHETIC MEDIUM.

Exp. Cell Research., 16: 118.

TROWELL, O.A. (1965). LYMPHOID TISSUE IN CULTURE. IN: CELLS AND TISSUES IN CULTURE Vol. 2. (Ed). E.N. Willmer.

Acad. Press, London, New York.

- TROWELL, O.A. (1965). PERSONAL COMMUNICATION.
- TURNER, C.W. (1939). THE MAMMARY GLANDS. IN: SEX AND INTERNAL SECRETIONS. (Ed) E. Allen. London: Ballier, Tindall and Cox.
- TURNER, C.W. and GOMEZ, E.T. (1933). NORMAL DEVELOPMENT OF THE MAMMARY GLANDS OF THE MALE AND FEMALE ALBINO MOUSE. Res. Bull. Mo. Agric. Exp. Sta., 182: 3.
- VOLLMAR, H. (1939). UBER DEN EINFLUSS VON VITAMINEN AUF GEWEBEKULTUREN VON NORMAL UND TUMORGEWEBE. Arch. exp. Zellforsch., 23: 42.
- WADE, N.J., and DOISY, E.A. (1935). CORNIFICATION OF VAGINAL EPITHELIUM OF OVARECTOMIZED RATS PRODUCED BY SMEARING. Proc. Soc. Exp. Biol. and Med., 32: 707.
- WARAVDEKAR, S.S. and RANADIVE, K.J. (1962). BIOLOGICAL STUDY OF A LINE OF SWISS MICE. Indian J. med. Res., 50: 175.
- WELLINGS, S.R., DE OME, K.B., and PITELKA, D.R. (1960). ELECTRON MICROSCOPY OF MILK SECRETION IN THE MAMMARY GLAND OF THE C3H/Org1 MOUSE. 1. CYTOMORPHOLOGY OF THE PRE-LACTATING AND THE LACTATING GLAND. J. Nat. Canc. Inst., 25: 393.
- WILLMER, E.N. (1965) (Ed) CELLS AND TISSUES IN CULTURE. METHODS, BIOLOGY AND PHYSIOLOGY. Vol. 1. Acad. Press. London, New York.
- WRIGHT, J.H. (1902). A RAPID METHOD FOR THE DIFFERENTIAL STAINING OF BLOOD FILMS AND MALARIAL PARASITES. J. Med. Res., 1: 138.
- YOSHIDA, M. (1963). STRAIN DIFFERENCES IN THE SENSITIVITY OF MAMMARY GLAND OF IMMATURE MICE TO ESTROGEN. J. Faculty Agric. Shinsku Univ., 3: 113.
- ZAKREWSKY, (1929). UBER DEN EINFLUSS VON NORMALGEWEBEN, INSBEDONDERE VON GEWEBEN ENDOKRINER DRUSEN, AUF DAS WACHSTUM VON JENSEN-SARKOMZELLEN IN VITRO. Zeitschr. f. Krebsf., 30: 123.
- ZIMMERMANN, K.W. (1898). BEITRAGE ZUR KENNTNIS EINIGER DRUSEN UND EPITHELIEN. Arch. mikr. Anat., 52: 552.
- ZIMMERMANN, K.W. (1927). IN VON MOLLENDORFFS HANDBUCH DER MIKROSKOPISCHE ANATOMIE DES MENSCHEN., 2: 130.
- ZUCKERMANN, S. (1932). QUOTED BY FOLLEY ET AL (1939).